



# Animal Cognition Meets Ecosystem Ecology: the Impact of Cognition on Seed Dispersal

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# Animal Cognition Meets Ecosystem Ecology: the Impact of Cognition on Seed Dispersal

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# Abstract

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Seed dispersal by endozoochory is important for the maintenance of plant populations and biodiversity. As a result, understanding the impact that frugivores' activities have on seed dispersal is essential in order to better understand plant population dynamics. One factor that is known to affect an animal's behaviour, yet has received little attention in this context, is animal cognition i.e. whether the information animals learn and remember affects where they access fruit and deposit seeds. Therefore, the aim of this thesis was to address how animal learning and memory affects the seed dispersal process, using two key approaches – experimental tests of frugivore cognition, and a model parameterised to examine the consequences of different cognitive abilities on seed dispersal. Three questions were investigated:

(1) The “where?” - whether the ability of frugivores to relocate previously visited food sources impacts upon their movements and, as a consequence, on plants' seed shadows. The spatial learning and memory of red-footed tortoises was tested using an egocentric task. Tortoises were able to navigate efficiently in the environment, and remembered the spatial location of food for at least two months. A seed dispersal model designed to test whether frugivores with different spatial memory skills differently affect plants' seed shadow, suggested that animals with long spatial memory relocate more efficiently food sources than animals' with shorter memory. As a consequence, animals with longer memory survived longer, dispersed a bigger amount of seeds, and moved less at random around the environment, all of which lead to different spatial distribution of deposited seeds.

(2) The “What?” - whether seed dispersers' discriminatory skills and memory affects their choice of fruit. Tortoises' ability to discriminate between quantity and quality of food was tested. They were able to successfully discriminate between the visual cues indicating different types of food and remembered the task for at least 18 months. A seed dispersal model designed to

investigate whether the memory of quality and quantity of food affects seed dispersal showed that the ability to discriminate between the features of fruits and, in particular, the memory of those, allows animals to base their foraging decision on previous learned experiences, significantly increasing the amount of seed dispersed from the preferred fruit.

(3) The “When?” - whether the ability to anticipate events, such as food availability, and learn about plants fruiting cycles affects plants’ seed shadows. Tortoises’ anticipatory skills were tested on a 24 h cycle. They were able to anticipate food delivery, showing an increase in activity immediately prior the scheduled food delivery time. A seed dispersal model designed to test the impact of timing on dispersal showed that animals that are able to anticipate cycles equal to or longer than plants’ fruiting cycle readily relocate food, survive longer and disperse more seeds than those with memory that doesn’t last as long as a fruiting cycle.

Finally, I present a model parameterised with red-footed tortoise cognitive data, with the aim to test one of the characteristics that makes tortoises unusual as seed dispersal vectors: the use of gaps in the forest. The results suggest that the active use of gaps enhances the probability of seed deposition in gaps and deforested areas, making tortoises a possible reforestation “tool”.

I have demonstrated that the study of frugivores’ cognition can help to build more reliable predictions of seed dispersal by endozoochory: cognition is probably the most effective way to understand and predict an animal’s choices and movements around the environment. Future research should incorporate cognition in the study seed dispersal via endozoochory to have more reliable predictions of plant dynamics.



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## *Declaration*

I declare that no material contained in the thesis has been used in any other submission for an academic award at this or any other institution.

I declare that the thesis is all my own original work, except where otherwise indicated.

## *Conference presentations/proceedings:*

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## ***Publications plan and authors' contribution.***

1. Soldati, F., John, L., Pike, T., Burman, O., Wilkinson, A. “Relative memory of food quantity in red-footed tortoise”.

This paper will include the results of experiment 3 and 4 described in chapter 3.

### *Authors' contribution:*

Experimental design: F.S.

Experimental design revision: A.W., T.P., O.B., L.J.

Experiment execution and data collection: F.S.

Data analysis: F.S.

Paper writing: F.S.

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This paper is meant to be a review, which underlines how the cognitive skills of animals can benefit the seed dispersal process. It will include part of the concepts explained in the introduction.

### *Authors' contribution:*

Paper writing: F.S. and L.J.

Paper revision: A.W., T.P., O.B.

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### *Authors' contribution:*

Model design: T.P.

Model partial modifications and application: F.S.

Data analysis: F.S.

Paper writing: F.S.

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Experimental design: F.S.

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# CHAPTER 1

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## 1. GENERAL INTRODUCTION

### 1.1 Seed dispersal definition, importance and theories

Seed dispersal is defined as all the mechanisms that provide the movement and/or transport, of seeds away from the parental plant. More precisely, according to the definition of Howe and Smallwood (1982), a *propagule* is the unit of a plant that is actually dispersed, and dispersal is referred as the departure of a propagule from the parental plant. *Seed rain* is defined as the flux of seeds from reproductive plants, without considering space explicitly (Nathan and Muller-Landau, 2000), while the spatial distribution of seeds dispersed from a single plant is referred as *seed shadow* (Nathan and Muller-Landau, 2000).

The consequences and benefits of dispersal to plants are many: (1) it is the only way to colonize new favourable habitat (Howe and Smallwood, 1982; Marsh *et al.*, 2004; Schupp *et al.*, 2010), (2) it can provide an escape from predation and competition, at the parental site (Nathan and Muller-Landau, 2000), (3) it affects the rate of gene flow (Ouborg *at al.*, 1999) and (4) it is the only possibility, other than adaptation, that organisms have to resist to climate change and habitat fragmentation (Ronce, 2001). The possibility of dispersal in different areas is essential to guarantee intra and inter-species diversity, decreasing the probability

of extinction of a species and maintaining ecosystem biodiversity (Frankham *et al.*, 2002, Leimu *et al.*, 2010). Thus, the seed dispersal process impacts on the structure and fitness of plant populations (Willson and Traveset, 2000).

For these reasons, seed dispersal is considered a key ecosystem process because it defines the future structure of plant communities (Howe and Smallwood, 1982). The resulting seed shadow, in fact, not only determines the potential area of plant recruitment, but it impacts on subsequent processes, such as predation, competition and reproduction of the future adult plants (Nathan and Muller-Landau, 2000). In a comprehensive review Schupp *et al.* (2010) showed that seed dispersal is central in many ecological aspects: understanding plants' gene flow (Jordano *et al.*, 2007), and metapopulation dynamics (Spiegel and Nathan, 2007), plant population dynamics (Jordano and Herrera, 1995; Schupp and Fuentes, 1995; Beckage and Clark, 2003; Ness *et al.*, 2006), community structure (Levin *et al.*, 2003; Levine and Murrell, 2003; Howe and Miriti, 2004), plant migration in response to historic and future climate change (Ibáñez *et al.*, 2006), evolutionary trade-offs (Clark *et al.*, 2004), structure of interaction networks (Bascompte and Jordano, 2007), maintenance of biodiversity (Bascompte and Jordano, 2007), structuring of species–area curves (Rosindell and Cornell, 2009), ecological consequences of habitat fragmentation (Cordeiro and Howe, 2003), weed invasions (Buckley *et al.*, 2006), ecological restoration (Nuttle and Haefner, 2007), the effectiveness of corridors for conservation (Levey *et al.*, 2005), and more. Because of the many ecological questions touched by seed dispersal, the awareness of the importance of this process for the ecosystem is in constant growth.

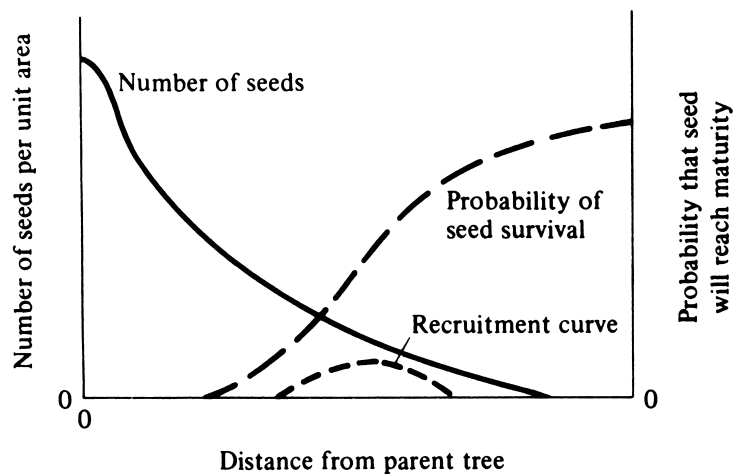
However, seed dispersal is an extremely complex process and it comes with several costs to the plant (Bonte *et al.*, 2011) in terms of energy, time, risk and opportunity (D' hondt, 2011). The energetic cost refers to the expenditure for the construction of special dispersal organs and tissue, such as wings for wind seed dispersal or fleshy fruits for endozoochory (Bonte *et al.*, 2011). Time cost refers to the time invested in dispersal that cannot be invested in other activities and the possibility of longer dormancy period that seeds might encounter in deposition sites (Bonte *et al.*, 2011); the cost of risk encompasses the risk of mortality and damage that seeds might suffer at each stage of the seed dispersal process, and the cost of opportunity refers to the possible loss of the advantages of the natal environment when colonising a new one (D'hondt, 2011; Bonte *et al.*, 2011).

### 1.1.1 Seed dispersal theories

Three main theories attempt to explain the principal reasons why seed dispersal has evolved, and which are the conditions that improve seed dispersal outcome, i.e. increased probability of seed survival and seedling establishment: the “escape hypothesis”; the “colonization hypothesis”; and “the directed dispersal hypothesis”.

1. **Escape hypothesis:** this first hypothesis, also known as Janzen-Connell hypothesis (Janzen, 1970; Connell, 1971), suggests that the survival rate of seeds increases proportionally with the distance from the parental plant at which the seed is deposited. The higher mortality near the parental plant is due to: (1) the higher chances for seeds to be eaten by seed predators, such

as rodents, insects, birds and herbivores, that tend to live close to the parental trees, and (2) to the higher seed density encountered under parental trees. However, at increasing distance from the parental tree the number of seeds deposited decreases (fig 1.1). Thus, the probability of seed establishment (recruitment curve in fig 1.1) is the highest at the distance that falls between the interception of the number of seeds and probability of seed survival curves (fig 1.1) (Janzen, 1970).



**Figure 1.1** Hypothetical model of seed recruitment versus distance from parental tree. Near the parental tree the probability of survival is null because of high seed density and seed predators. At increasing distance from the parental tree the probability of seeds survival increase, as seed density and seed predator decreases. However, the number of seeds deposited drops, so that the recruitment is maximum between the interception of the number of seeds and probability of survival curves (figured adapted by <http://www.zo.utexas.edu/courses/bio301/chapters/Chapter15/Chapter15> from Janzen's original paper (1970))

This hypothesis is widely accepted and many field studies, primarily focused on young seedlings, have demonstrated increasing survival with increasing distance from parents, as genetically related seedlings compete more than seedlings originating from different parents (Ellstrand and Antonovics 1985 in Wilson and Traveset, 2000; McCall *et al.*, 1989), or



decreasing density of propagules, as competition among seeds is lower at lower density (e.g. Gilbert *et al.*, 1994; Harms *et al.*, 2000; Swamy and Terborgh, 2010; Schupp and Jordano, 2010). The high mortality of the offspring near its parental trees opens up spaces for other plant species that otherwise would be excluded by the intense competition from the seeds of nearby plants (Howe and Miriti, 2000). This results in the enhancement of species coexistence and species richness (Harms *et al.*, 2000; Schupp and Jordano, 2010). Another way plants have to escape predation is dispersal in time (Guzmán and Stevenson, 2011). This refers to the mechanisms that cause seeds to be deposited with a delay in relation to the fruiting period of a plant. Seed dispersers that have long gut passage time provide this, such as chelonians, which have a gut retention time that ranges between 4 and 28 days (Strong, 2005). Seed dispersal in time was demonstrated to decrease the risk of density-responsive predation that occurs during the fruiting period (Guzmán and Stevenson, 2011).

2. **Colonization hypothesis:** this hypothesis suggests that occupying new, favourable habitats, might be the major advantage of seed dispersal (Howe and Smallwood, 1982). This is because (1) parental plants have low chances to replace themselves in situ for the reasons explained in the escape hypothesis, and (2) seeds that are disseminated as widely as possible increase their chances of finding new areas with optimal conditions and less competition, thus increasing plants' fitness. This strategy is thought to be advantageous, for example, for plants whose requirements for establishment are met in scattered locations, such as tree-

fall gaps or fallen logs (Sargent, 1995; Wilson and Traveset, 2000) in comparison to less “exigent” plants, such as shade tolerant plants. However, there are no direct tests of this hypothesis in forests, but it has been explored experimentally (Clark *et al.*, 2004). Results showed that seeds’ colonization widely varies among species and also between individuals of the same species, making difficult to confirm this hypothesis (Clark *et al.*, 2004).

3. **Directed dispersal hypothesis:** this hypothesis is valid for dispersal via zoochory only, as it suggests that plants aim to ensure that diaspores reach deposition sites especially suitable for establishment and survival (Howe and Smallwood, 1982; Wenny, 2001). Thus, it requires that dispersal agents (animals) take the seeds to non-random places (Howe and Smallwood, 1982). Typical examples of directed dispersal are given by scatter-hoarding animals (Vander Wall, 2010), some species of fruit eating birds (Wenny and Levey, 1998; Wenny, 2001), ants (Ohkawara *et al.*, 1997) and frugivorous reptiles that use treefall gaps (see chapter 5). However, plants rely on more than one animal, and often on more than one dispersal agent to ensure dispersal of their seeds, suggesting that directed dispersal is not the primary advantage of seed dispersal (Wenny, 2001).

Of course, these hypotheses are not mutually exclusive. According to the species and the environmental situation under investigation, one can be more advantageous than the other, but it is likely that most of the plants benefit from all

of them (Wenny, 2001). Seeds must be dispersed in order to increase plants fitness and plants would be better benefitted by dispersal agents that provide long dispersal distance in favourable sites. Thus, the effectiveness of seed dispersal, i.e. seed dispersal events resulting in the successful establishment of new individuals (Schupp, 1993), relies mostly on the mechanism and dispersal vector adopted by the plant (Schupp *et al.*, 2010).

## **1.2 Mechanisms of seed dispersal**

In order to achieve effective dispersal (*sensu* Schupp *et al.*, 2010), plants have evolved different seed dispersal mechanisms, which involve different dispersal vectors; these can be abiotic, such as wind and water, and biotic, such as animals (Nathan *et al.*, 2008). According to the strategy adopted, seeds have evolved different characteristics to better serve the purpose. To follow, a brief description of the seed dispersal mechanisms, particular attention will be given to seed dispersal by endozoochory as it is the process this thesis is focused on.

### **1.2.1 Explosive seed dispersal**

Explosive seed dispersal is the mechanism according to which seeds are discharged by the rupture of the fruit due by elastic contractions of its tissues (Garrison *et al.*, 2000). This mechanism is regulated by humidity and temperature; when the pod containing the seeds dries, it breaks releasing the seeds in the environment. This mechanism is used for example by plants of the family *Geraniaceae*, *Fabaceae* and *Brassicaceae*. The distance at which the seeds are

launched after the explosion can reach up to half a metre (Evangelista *et al.*, 2011). Thus, a relatively small distance compared to other dispersal means (see below). Nonetheless, it is common that plants that primarily use explosive dispersal also rely on insects, in particular ants, for secondary dispersal, ensuring that the seeds are transported farther away and buried in an appropriate microsite (Willson, 1993; Narbona *et al.*, 2005). The advantage of explosive dispersal is that the plant does not require a primary dispersal vector, however, the seed dispersal distances reached by this process are very short in comparison to the ones reached by other mechanisms explained below.

### **1.2.2 Seed dispersal by abiotic agent: wind and water**

Seed dispersal by abiotic agents is a mechanism adopted by vast range of plants, from grasses to trees. It depends mainly on winds and/or currents of water. This kind of dispersal can lead to longer dispersal distance compared to the explosive strategy, however the range of distances that could be reached vary significantly according to the configuration of landscape and meteorological conditions encountered, thus abiotic vectors might be more efficient in certain types of environments than others (Nathan *et al.*, 2008). Seeds dispersed by wind are often light and may have wing-like or hairy structures that allow them to be lifted and pushed by air. A typical example is give by the dandelion seeds (Hampe, 2004). Ocean currents appear to be the most efficient water dispersal vector in terms of distance. Although this process might take a very long time (from days to months), it can transport seeds at hundreds of kilometres of distance from the starting point. This mechanism requires seeds to have structures that

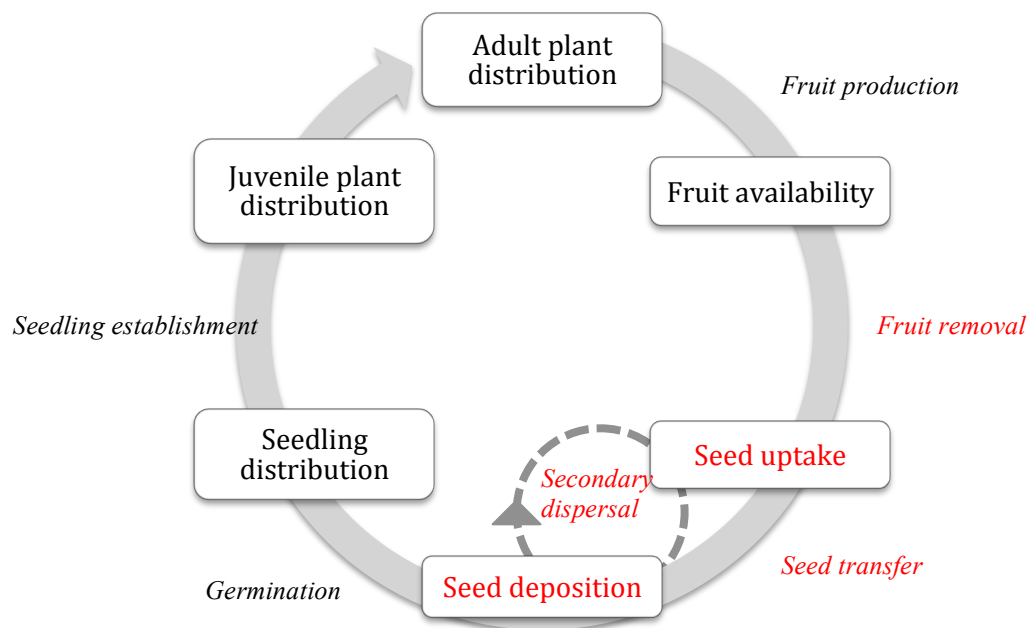
allow them to float and to survive for months in water. A typical example is given by coastal plants, such as the coconut tree (Thiel and Haye, 2006).

Plants using abiotic dispersal vectors may benefit of long dispersal distances, which depend on the structures of the seed and the strength of the flow, however, currents and winds often maintain a consistency in their flow, consequently decreasing the chances of plants to colonize new areas, and increasing seed deposition in high seed density areas (Nathan *et al.*, 2008).

### **1.2.3 Seed dispersal by biotic agent: animals**

Seed dispersal by animals (zoochory) is probably the most complicated dispersal mechanism studied. Unlike dispersal by abiotic factors, zoochory relies on animal behaviour, which results from internal and external stimuli that might be difficult to predict (Russo *et al.*, 2006; Cousens *et al.*, 2010). Zoochory mechanisms can be classified into two main categories: epizoochory and endozoochory. The first refers to the transport of the seeds on the outside of vertebrate animals. Seeds that adopt this mechanism are well exposed on stems and tend to have hairs and spikes able to anchor themselves to the fur and hooves of mammals (Couvreur *et al.*, 2004a). This allows seeds to be transported and deposited at distances that vary according to the disperser dimensions and the adhesive propriety of the seed itself, but that can commonly reach 100 meters from the parental plant (Couvreur *et al.*, 2004b; 2008). Endozoochory refers to the transport of the seeds inside vertebrates, thus it requires the disperser to ingest the seed. Plants in the tropical regions mostly adopt this seed dispersal mechanism, in fact between 50 to 90% of shrubs and trees species rely on fruit-eating vertebrates

for dispersal (Fleming *et al.*, 1987). This process requires an active uptake of the seeds by the dispersal vector and, to be successful, the seeds have to survive three key stages: uptake, transport (in the gut) and deposition (fig 1.2). Each stage of this process encompasses costs for plants, which are described in table 1.



**Figure 1.2** Cycle of seed dispersal by endozoochory. The boxes indicate seeds dispersal pattern, while the processes are indicated in italic font. In red are indicated the stages that are mainly controlled by animals. The dashed line indicates the possibility of secondary dispersal by a different dispersal vector (figure adapted from Wang and Smith, 2002)

**Table 1 Costs of the seed dispersal by endozoochory (adapted from Bonte *et al.*, 2011)**

Phase	Cost	Causes
Fruit production	Energetic cost	Metabolic investment in the production of fleshy fruits.
Fruit removal	Risk cost	Mortality and damage due to fruit predation.
Seed uptake	Risk cost	Seed mortality during ingestion due to animals' chewing.
Seed transfer	Risk cost	Seed mortality due to animals' digestive process.
Seed deposition	Opportunity and risk cost	Mortality due to poor quality habitat. Post transfer predation.
Germination	Time and risk cost	Possible longer dormancy period because of the conditions of the new area. Herbivory on young seedlings.
Seedling establishment	Opportunity cost	Lesser habitat quality

#### *1.2.3.1 Uptake: finding and eating the fruit*

The first step of endozoochory is the ingestion of the seed by the animal. At this stage it is essential that plants attract and motivate dispersal vectors to ingest their seeds (this topic will be extensively discussed in section 1.5.2 and chapter 3). To this purpose, seeds are wrapped in a nutritious and fleshy product, the fruit, often externally characterised by bright colours and scents. The pulp of the fruit contains nutrients such as water, fat, sugar, protein and minerals, thus it represents an excellent food source for animals (Fleming and Kress, 2011; Cazetta *et al.*, 2009), at a level that some species are now defined as frugivores, as their diet is composed of more than 70% fruit. This category includes species of tropical birds, mammals, and reptiles. Seeds can have different shapes and sizes

and they are commonly protected by a lignified layer endocarp. During uptake, seeds may encounter their first cause of mortality due to animal chewing. Mortality rate depends on both seeds (characteristics of the endocarp) and animal species (teeth) and can range from 0 to more than 50% (Traveset, 1998; Cosyns *et al.*, 2005). At this stage, seed dispersal effectiveness, i.e. the actual number of seeds that will successfully establish, is generally enhanced by (1) an abundant seed uptake, because the bigger the number of seeds dispersed, the bigger are the chances that part of them would end in favourable areas (Schupp *et al.*, 2010), (2) a fast seed removal, as it decreases the chances that fruit would be eaten by seed predators or remain under the parental tree (Schupp *et al.*, 2010) and (3) low mortality due to animal chewing.

#### *1.2.3.2 Transport*

The transport of the seed by endozoochory is carried out inside the gut of the seed disperser. This phase determines the dispersal distance from the parental plant (e.g. Cain *et al.*, 2000; Jordano *et al.*, 2007; Nathan *et al.*, 2008). As we saw in the previous section (1.2), seeds increase their chances of survival at increasing distances from the parental trees, in particular if they are deposited at a distance where the recruitment is the highest (see figure 1.1, section 1.2) (Janzen, 1970). The dispersal distance is calculated by the gut retention time multiplied by the daily distance travelled by the animal (Nathan *et al.*, 2008). Therefore, the most efficient disperser would, hypothetically, be an animal with slow digestion, that travels long distances every day. However, there is a trade-off between the gut passage time and the viability of seeds after gut passage (Traveset, 1998): the



longer the seeds stay in the gut, the greater are the chances that seeds would be damaged by digestion processes. Although many factors contribute to seed viability after gut passage, including the features of the digestive tract of the disperser, the characteristics of the seeds (e.g. thickness and size of seed coat), the amount and type of food ingested along with the fruit (see Traveset, 1998 for a complete review); the rate of seed viability after defecation is commonly used to measure the efficiency of the seed disperser (Schupp *et al.*, 2010). The distance travelled by frugivores varies substantially among species (e.g. Jordano *et al.*, 2007; Wotton and Kelly, 2011) and is impacted by the distribution of resources in the environment, as animals tend to be attracted and spend longer time in areas where the resources are abundant (Russo *et al.*, 2006). The quality of the movement, other than the distance, is also important, as it affects the shape of the seed shadow of trees and the local density of seeds: for example, an animal that is very active, but moves always in the same area, would provide a smaller and more dense seed shadow than an animal that is less active but moves in many directions (Wescott and Graham, 2000; Clark *et al.*, 2005; Di Fiore and Suarez, 2007). How frugivores' movements affect seed dispersal will be discussed in more in depth in section 1.5.4.

#### *1.2.3.3 Deposition*

The site where the seed is deposited is important, as it influences the chances of seedling establishment and future fitness of the plant (Shupp *et al.*, 2010). The microsite is defined as an environmental area suitable for the seed establishment of a given species (Nathan and Muller-Landau, 2000). The quality

of the deposition site depends on to the distance from a conspecific, habitat variability and competition with neighbouring plants (Schupp *et al.*, 2010). It could range from small scale, for example the advantages received from seed burial (Strong and Fragoso, 2006; Vander Wall, 2010), to large scale, as the consequence for dispersal in close canopy in comparison to gaps in the forest (Herrera *et al.*, 1994; Wenny and Levey, 1998). The success of this phase depends mostly on the animals' use of the environment and/or its defecation behaviour (Nathan *et al.*, 2008). Tapirs (*Tapirus terrestris*), for example, defecate in dedicated latrines, resulting in areas far from the parental trees where seed density is high (Fragoso *et al.*, 2003). Reptiles tend to use forests gaps more than other frugivore species, because of their thermoregulatory needs (Greenberg, 2001) favouring the establishment of pioneer species (Popma *et al.*, 1988). However, despite the numerous studies on seedling survival and germination after gut passage time in laboratory settings (e.g. Traveset *et al.*, 2001; Cosyns *et al.*, 2005; Mancilla-Leytón *et al.*, 2011) precise data in nature on post-dissemination stages of seed dispersal are lacking. Thus, the link between seed dispersers' activity and the actual quality of deposition sites is not clear (Schupp *et al.*, 2010).

#### *1.2.3.4 Diplochory: seed dispersal does not stop with the primary vector*

In the previous sections, the principal mechanisms that plants adopt to disperse their seeds have been briefly explained. However, even if they are generally divided into three disjointed categories, studies have shown that this should not be the case: the seeds' complete journey does not always end after the primary dispersal vector, and, in most cases, it relies on more than one mechanism

(e.g. Nathan *et al.*, 2008; Traveset *et al.*, 2014). Seeds primarily dispersed by wind can successively be transported by water (Hampe, 2004) or animals (Tackenberg *et al.*, 2006). Seeds transported by endozoochory can receive a second passage from animals that feed on faeces of the primary disperser, such as insects (Christianini and Oliveira, 2010). All the mechanisms that provide secondary seed dispersal go under the definition of diplochory (for a complete review see Vander Wall and Longland, 2004). Although it is a topic that has received less attention than primary dispersal due mostly to logistical challenges (Traveset *et al.*, 2014), diplochory could reshape the seed shadow of trees increasing significantly the dispersal distance and transporting seeds to more favourable germination sites (Vander Wall and Longland, 2004; Traveset *et al.*, 2014). Ants and dung beetles, for example, tend to bury the seeds offering them more protection than if they were exposed on the soil (Vander Wall and Longland, 2004; Narbona *et al.*, 2005). Thus, diplochory should receive bigger attention in the future to complete the seed dispersal loop.

### **1.3 The importance of reptiles as seed dispersers**

Seed dispersal by reptiles, or saurochory, has received less attention than endozoochory processes performed by mammals and birds. This is the case (e.g. crocodile, Platt *et al.*, 2013), because reptiles are considered mainly carnivorous, e.g. only 1% of lizards are known to be totally herbivorous (Cooper and Vitt, 2002), thus their role in seed dispersal has been poorly investigated. However, recent investigations have shown that many species of reptiles complement their diet with nectar and fruits: 34 species of lizards (Olesen and Valido, 2003,

Godínez-Álvarez, 2004), 13 crocodylia species (Platt *et al.*, 2013), and one species of snake (Engel, 1997) have been observed eating fruits. Chelonians are an exception among Reptilia, since 25% of them are recognised as herbivorous (King, 1996 in Olesen and Valido, 2003) thus, they have received more attention as seed dispersers. It is believed that reptiles played an essential role as dispersers for the first angiosperms with fleshy fruits that appeared on earth, as mammals were still not present, and nowadays it is estimated that saurochory represents the 1% of all endozoochory (Olesen and Valido, 2003). Therefore, it is a topic that deserves attention, in particular, for two aspects that make this mechanism very peculiar: (1) studies revealed that saurochory plays an essential role in environment where the big dispersers are missing, e.g. in insular contexts (Olesen and Valido, 2003). A recent study on seed disperser-plant interactions in the Galapagos, for example, showed that reptiles were surprisingly important in this role, as the 50% of plant-reptiles interaction lead to seed dispersal events, in comparison with the 16% reported for birds (Heleno *et al.*, 2011). (2) Reptiles are able to provide excellent seed deposition microsites. They are ectothermic animals, thus they are forced to visit places exposed to the sun, such as tree-fall gaps in the forest, and they tend to rest in cover shelters under rocks or dead wood. Both conditions are excellent for the seeds because in the first case they would have the advantage of solar radiations; in the second case seeds would receive protection from grazing herbivores.

### 1.3.1 Seed dispersal by snakes

Seed dispersal by snakes is a phenomenon that has not been well documented. From the few studies that have reported snakes eating fruits (see Engel, 1997), it seems like direct ingestion of fruits is triggered by the infestation of the pulp by insect larvae (Mookerjee, 1946; Pope, 1961 in Engel 1997). Otherwise, the presence of fruit in snakes' stomachs, might be due simply to the ingestion of prey that had been feeding on fruit, thus it is possible that snakes act indirectly as secondary dispersers. Foraging habits apart, a factor that might represent a problem for the seeds ingested by snakes is the extremely powerful digestive juices in their stomach. Engel (1997) addressed the question of whether seeds are able to survive gut passage in snakes. He fed five snakes of four different species (*Rhamphiophis rubropunctatus*, *Rhamphiophis oxyhyncus*, *Naja mossambica*, *Bitis arietans*) with dead rats containing a known amount of seeds. Then he analysed the seeds in the faeces and he surprisingly found that two of the five species of seeds fed to the snakes, one hard coated and one soft coated seed, were viable. However, the germination time was longer than if the same seeds were ingested by bushbaby or planted directly from the fruits. The gut passage time ranged from two to 14 days, as found for most of reptiles. Thus, snakes can potentially contribute to seed dispersal, however the extent and how often this phenomenon would happen in nature is unclear. Further investigation is needed to clarify and evaluate the role of snakes as seed dispersers.

### 1.3.2 Seed dispersal by crocodilians

A recent review on the foraging habits of crocodilia was made in 2013 (Platt *et al.*, 2013). In this review the authors included information collected from papers and from field data regarding the stomach contents of the American alligators (*Alligator mississippiensis*) given by the Luisiana Department of Wildlife and Fishery. Results showed that the 72.2% of crocodylia species, had fruits in their diet. In the majority of cases, data did not report if the individuals ingested directly the fruit, or if the presence of seeds in their stomach and faeces was the results of a secondary ingestion as in the case of snakes (Nogales *et al.*, 1998; Platt *et al.*, 2013).

However, Platt and colleagues hypothesized that alligators might ingest seeds on purpose, for the same reason that they ingest small rocks. There was no evidence of frugivory in crocodylia, however, the authors suggested that these reptiles could potentially act as good seed disperser as they do not chew the food (Cleuren and De Vree, 2000), which would result in no damage for seeds during ingestion process, and their daily movements can be extensive. This, combined with a gut passage time above four days on average (Davenport *et al.*, 1990), would provide long dispersal distances, but no real data are available to verify these assumptions. Faeces were found at basking sites, in particular, *Crocodylus siamensis* faeces were found along seasonally exposed lake margins and on mats of floating vegetation (Casas-Andreu and Quiroz 2003), all of which represent good deposition microsites. All these characteristics would make crocodylia excellent seed dispersers. However, the pH of their stomach is highly acidic and the muscular action of the stomach walls is strong, thus the condition of seeds

after gut passage is uncertain. A recent study (Rosenblatt *et al.*, 2014) looked particularly into this matter, investigating seeds of pond-apple tree viability after gut passage in the stomach of *Alligator mississippiensis*. Results were not positive, as seeds were found to be incapable of germination, suggesting that alligators act like seed predators rather than seed dispersers. However, one study is not enough to evaluate the role that these reptiles play on seed dispersal. Seeds of different species should be tested, before drawing definite conclusions.

### **1.3.3 Seed dispersal by lizards**

The majority of the studies on seed dispersal by lizards, and saurochory in general, takes place on islands (Olesen and Valido, 2003). Populations of lizards on islands are found to have bigger fruit and nectar components in their diet, compared to lizards on the mainland (Olesen and Valido, 2003). It has been hypothesized that this is due to the combination of two phenomena: the scarcity of arthropods and lower predation risks (Olesen and Valido, 2003; Gódinez-Alvarez, 2004). The shortage of insects in insular ecosystems might have driven competition for food between lizards forcing them to expand their diet (the same situation has been observed in desert environment (Brown, 1991; Whiting and Greff, 1997). At the same time, it is generally recognized that frugivory and/or herbivory are associated with larger body size, because of the adaptations of the digestive tract necessary to digest plant material (Cooper and Vitt, 2002, Herrel *et al.*, 2004). Larger body equates to bigger visibility to predators, but in environments where predators are almost absent this increase in size does not represent a substantial risk for the animal. Moreover, the fact that on islands there

are no native mammals and fewer birds in comparison to the mainland, has opened a niche that has been occupied by these reptiles (MacArthur *et al.*, 1972).

In general, studies on frugivorous lizards agree that they represent good seed dispersers; these reptiles ingest a good amount of seeds of different plants, cause little or no damage to the seeds during the ingestion and digestion processes, and provide good deposition microsites. More rare are data on lizard movements, which leaves uncertain their contribution for long distance dispersal (Valido and Nogales, 1994, Nogales *et al.*, 1998, Godinez-Alvarez, 2004, Nogales *et al.*, 2012). Just to give some numbers, a study on 441 faeces of the lizard *Gallotia galloti*, in the Canary Island, revealed that they contained 1526 seeds. According to the plant species, seeds experienced low or no effect at all on their germination rate after gut passage, with less than 3% of damaged seeds reported (Valido and Nogales, 1994).

#### **1.3.4 Seed dispersal by chelonians**

As already mentioned, chelonians have received more attention in the evaluation of their contribution as dispersers, because of the substantial number of species, on either islands and mainland, that are known to be highly frugivorous or herbivorous. In many ecosystems the interaction of turtles with plants is so important that they have gained the title of “ecosystem engineers” (Griffiths *et al.*, 2010). For example, the gopher tortoise *Gopherus polyphemus* contributes to seed dispersal, nutrient cycling and maintenance of habitat heterogeneity of the North American grasslands and forests (Birkhead *et al.*, 2005; Means, 2006); and terrapins (*Rhinoclemmys funerea*) are important in the seed dispersal of aquatic



plants (Moll and Jansen, 1995). On islands the dispersal services of tortoises are so fundamental that the areas where they have become extinct are facing serious problems of habitat loss. For example, on the Mascarenes Islands, the loss of the giant tortoise *Cylindropsis sp.*, has caused the degeneration of the native grassland community and plants with large fruits have suffered a decrease in dispersal and fitness (Griffiths *et al.*, 2010). To overcome the lack of dispersal, non-indigenous tortoises (*Aldabrachelys gigantea* and *Astrochelys radiata*) with similar habits to the extinct indigenous ones were introduced in a process defined as “taxon substitution” (Hansen *et al.*, 2010), with an attempt to recreate the lost interaction and restore lost vegetation. The introduced tortoises helped to restore the endemic fauna by grazing preferentially on the faster growing exotic plants. This reduced the reproduction of faster growing plants and, as a consequence, the endemic plants had less competition and time to re-establish. Moreover, tortoises consumed and dispersed the large seeds whose dispersal had been limited since the extinction of the previous species (Griffiths *et al.*, 2010). This attempt at species translocation is not an isolated case; a similar approach has also been employed in Indian Ocean Island (Jones, 2002) and in North America, but with the attempt to re-establish the indigenous tortoise population (Tuberville *et al.*, 2005).

#### *1.3.4.1 Tortoises as dispersal vectors in tropical environments: the role of the red-footed tortoise*

Frugivorous tortoises are considered important seed dispersers in tropical environments (e.g. Moll and Jansen, 1995; Varela and Bucher, 2002; Liu *et al.*,

2004, Strong and Fragoso 2006, Jerozolinski *et al.*, 2009, Blake *et al.*, 2012). I will discuss the reasons why, by focusing on the red-footed tortoise (*Chelonoidis carbonaria*), as it is the experimental model used in this thesis, but they can be applied to other tropical species (e.g. *Chelonoidis denticulata*, *Rhinoclemmys annulata*).

The red- footed tortoise inhabits the South American forest from Panama to the east part of Brazil. Despite its highly frugivorous diet, it has received little attention as potential seed disperser. To date only two studies have examined its role as seed disperser, one in 2006 by Strong and Fragoso and the other in 2011 by Wang *et al.* From these studies the judgement of red-footed tortoise as a disperser is positive. As first, tortoises do not chew their food. This means that they swallow pulp and entire seeds without damaging them. Moreover, tortoises' digestive processes do not damage seeds: studies reported between 90 and 100% of viability of seeds in the faeces of red-footed tortoise (Strong and Fragoso, 2006, Wang *et al.*, 2011). They eat a variety of different fruits at the same time (Moskovitz and Bjorndal, 1990). This results in faeces presenting a mixture of seeds of different species that will be deposited together, reducing the intra-specific competition during the establishment phase of seedlings. Tortoises feed on the ground, thus they help remove fruits discarded by other frugivores species, such as birds and monkeys (Strong and Fragoso, 2006), that otherwise would suffer a higher risk of mortality due to the proximity of the parental tree (see 1.2). More importantly, red-footed tortoises provide also secondary dispersal of seeds as their diet includes the faeces of other dispersers (Moskovitz and Bjorndal, 1990; Merchan *et al.*, 1998). Thus, they participate to re-shape the seed shadow of trees. As explained in the section 1.2, essential for the quality of seed dispersal, is

the distance at which seeds are going to be deposited from the parental plant. For this to be maximized, seed dispersers should travel long distances and have, ideally, a long gut retention time. Red footed tortoises do not excel in activity and daily movement, their average movement is between five to 22 meters per hour, but there have been documented cases in which tortoises travelled at 200 m per hour (Moskovitz and Kiester, 1987). However, they possess a very slow metabolism, on average four days (Strong and Fragoso, 2006), that allows tortoises to disperse seeds at an average distance of 100m from the parental tree, with peaks calculated around 600m (Strong and Fragoso, 2006). In addition, the length of the gut passage of the tortoises provides dispersal in time, decreasing seed predation risk (Guzmán and Stevenson, 2011). Tortoises spend their resting time hidden in tight burrows covered by leaves, preferably in tree-fall gaps, or under dead wood. They use these places as shelter also when they are flooded and muddy (Moskovitz, 1985). These conditions make these sites excellent deposition microsites, where the chances of seed establishment are high (Strong and Fragoso, 2006). Often, more than one tortoise uses the same burrow; this contributes to enhance the variety of seeds deposited in the same site (Moskovitz, 1985). Like all the reptiles, red-footed tortoise are ectothermic animals, thus they need an external heat source to regulate their body temperature. For this reason they spend time in forest gaps, which are rarely used by other seed dispersers, such as birds and mammals, because they are open areas where the predation risk is higher. Gaps are excellent deposition microsites, in particular for gap-dependent pioneer species such as *Genipa Americana* (De Souza *et al.*, 1999), because they are the rare areas in the forest where the sunrays hit directly the soil (De Souza *et al.*, 1999; Strong and Fragoso, 2006).

In summary, reptiles cover an important role as seed dispersers, in particular for their peculiar use of tree-fall gaps and shelters that provides excellent deposition microsites for seeds. The attempts to define the role of reptiles as seed dispersers are substantially increasing, however, little is still known about two classes, snakes and crocodylia, while it seems well recognized that lizards, and in particular tortoises, provide essential dispersal services, especially in an insular context, where their services are not easily replaceable by non analogous animals.

#### **1.4 The idea: introducing animal cognition to the seed dispersal process**

In the previous sections, it was explained how the acquisition and transport of seeds suitable microsites is essential for successful endozoochory. To date, research on frugivores has focused mainly on their behavioural ecology, assessing their quality as dispersers on the basis of the number of fruit species eaten, the number of seeds processed, the amount of viable seeds contained in faeces and the estimation of long seed dispersal distance combining together data on animal daily movement and gut retention time (e.g. Schupp, 1993; Link and Di Fiore, 2006; Strong and Fragoso, 2006; Jerozolinski *et al.*, 2009). However, these studies tend to describe the seed dispersal phenomena without considering the cognitive skills behind the decision making process of the animals, i.e. whether what animals learn and remember impacts upon where they access fruit and

deposit seeds (Corlett, 2011). Animals are likely to use a multitude of cognitive skills for successful foraging: it would be adaptive to be able to learn about cues in order to identify fruits, to use remote environmental stimuli to locate potential food sources in time and space, to be able to discriminate between samples in order to select edible or better fruits in terms of quantity and quality. Further, short and long-term spatial memory is likely to be important to be able to relocate food sources. Thus, an integrative approach in the analysis of frugivores' cognitive skills may reveal impacts on seed dispersal otherwise difficult to discover, helping, for example, to predict animals' foraging decision or movement patterns. Therefore, this thesis aims to combine the ecological concepts of seed dispersal with a cognitive approach, in order to examine the impact that learning and memory play on frugivores' foraging behavior, and the consequent impact upon seed dispersal.

#### **1.4.1 Why what animals choose to eat impacts on seed dispersal**

Understanding frugivores' foraging behavior, in particular under choice situation, e.g. how they select fruits, is essential to comprehend the seed dispersal process. According to Schupp *et al.* (2010), the quality of a seed disperser, in terms of amount of seeds dispersed from an individual plant, is calculated by multiplying the amount of seeds that the animal removes every foraging bout by the number of times the animal visits the same plant. This implies that if animals are motivated to forage and return to feed from the same food source, then this would enhance the seed removal process and increase a plants fitness. But what

motivates animals to choose some fruits instead of others and to visit a particular plant multiple times?

#### **1.4.2 Food motivation and choice in animals**

In his recent review, Corlett (2011) described how seed dispersers might use a multitude of physiological (e.g. innate appetite for deficient nutrients) and cognitive skills in order to make their food choices. The preference for a fruit could be driven by its traits, such as colour, scent and shape. Many studies have devoted attention to how these traits can be used as indicators of nutrient content and ripeness of fruits (e.g. Schaefer *et al.*, 2003; Schaefer *et al.*, 2004; Cazetta *et al.*, 2009; Schaefer, 2011; Cazetta *et al.*, 2012) and cognitive studies have confirmed that animals possess the discriminatory skills - i.e. ability to detect specific features of an object in order to recognize and categorize it (Wilkinson and Huber, 2012) - to discriminate successfully on the basis of these cues (e.g. mammals discrimination of colours: Kelber, 2007; Dominy *et al.*, 2003; shape: Gosset and Roeder, 2000; smells: Hübener and Laska, 2001; and textures: Dominy *et al.*, 2001; visual discrimination in fish: Colwill *et al.*, 2005; visual discrimination in reptiles - brightness, colour and shape - reviewed in Burghardt, 1977; olfactory and visual discrimination in insects: Balkenius *et al.*, 2006). Thus, it is generally accepted that perceptual features of fruit play a fundamental role in attracting frugivores and are used by animals to select the best products (Schaefer *et al.*, 2004).

After eating a fruit and moving away from the food source, the decision to eat that fruit again could depend on its taste (Yarmolinsky *et al.*, 2009; Zhao *et al.*,

2010) and digestive feedback (Yearsley *et al.*, 2006; Werner *et al.*, 2008). However, this information it is not enough to explain what motivates an animal to choose certain fruits: in their natural environment. Frugivores show a very mixed diet (Moskovits, and Bjorndal, 1990; Guzmán and Stevenson, 2008) and it is difficult to find a correlation between fruit eaten and animals' fruit preferences reported in experiments (Carlo and Morales, 2008). To explain this, a first hypothesis states that frugivores respond to their nutritional needs (Murphy and King, 1987), tending to balance their nutrient intake, such as minerals, protein and vitamins (McCaughey and Tordoff, 2002). Thus, their choice of fruit would be driven by innate appetites for nutrients and not exclusively by their preferences (Murphy and King, 1987). A second hypothesis relies on the optimal foraging theory, according to which animals adjust their behavior in relation to the cost and benefits associated with a food source (Kacelnik and Houston, 1984; Pyke, 1984). In this scenario, the quantity and quality of food obtained at each foraging event (benefits) and other factors (costs), such as the proximity of the food source to the current position of the animal (Levey *et al.*, 1984), the ability of the animal to relocate the food source (Di fiore and Suarez, 2007), food accessibility, location in relation to other food sources and predation risk, can alter fruit preferences (Kacelnik and Bateson, 1996; Kacelnik *et al.*, 2013). This is supported, for example, by studies on tanagers (*Mitrospingus cassinii*, *Habia fuscicauda*, *Ramphocelus passerinii*, *Tachyphonus. delatrii*) and manakins (*Pipra mentalis*, *Manacus candei*) that change their likelihood of feeding on less preferred food when the distance between perches increases (Levey *et al.*, 1984); steller's Jays (*Cyanocitta stelleri*) that modify their food choice depending on the presence of other conspecifics in the feeding area (Bekoff *et al.*, 1999), and chaffinches

(*Fringilla coelebs*) that choose less cryptic seeds in environment where the predation risk is higher (Jones *et al.*, 2006). All these features affect animal motivation to return to certain food sources, thus, they should be taken into account when considering seed dispersal. Plants that offer the biggest benefits associated with the lowest costs are the most likely to receive more visits and have more seeds dispersed than plants with higher costs. Thus, when evaluating seed dispersal, both benefits and costs should be taken into account to have a more reliable prediction of the number of visits to a plant and the amount of seeds dispersed.

#### **1.4.3 Food quantity as motivation for food choice**

Food quantity is another important indicator of the nutritional value (benefit) of food source, however it has received less attention than quality as parameter for food choice in an ecological context. Together with quality, the amount of food gained by animals has proved to be one of the major factors that impact upon their growth and fitness (Pyke, 1984; Guisande, 2000; Cruz-Rivera and Hay, 2000). This implies that animals would be advantaged if they were able to determine food patches with the largest amount of food (Dehaene, 1997). The ability to discriminate between food quantities has been explored and demonstrated in a variety of taxa (mammals: Ward and Smuts, 2007; Beran, 2008; fish: Addessi *et al.*, 2008; birds: Al Aïn *et al.*, 2009; amphibians: Krusche *et al.*, 2010), although very little is known about this ability in reptiles. Most of the research on this topic has focused on numerical discrimination, an ability that many animals have been shown to possess (Agrillo *et al.*, 2007; Addessi *et al.*,



2008; Al Aïn *et al.*, 2009). Fewer studies have focused on the ability to discriminate between different volumes of food (Boysen *et al.*, 2001; Stevens *et al.*, 2007; Beran, 2008), but this skill is likely to be adaptive as a larger volume of food is potentially indicative of a higher amount of calories, and by choosing it, animals could maximise the mass of food obtained at one time (Mahamane *et al.*, 2014). Chimpanzees (*Pan troglodytes*), for example, are biased towards sets of food that include the largest item even if the total amount of food is smaller than the alternative sets of food offered to them (e.g. Boysen *et al.*, 2001; Beran, 2008). However, when the items were replaced by symbols, so that the subjects could not see the relative sizes, chimpanzee were able to choose the total biggest amount of food without being biased by its volume (Silberburg and Fujita, 1996). Beran (2008) suggested that this inclination towards larger items could be ancestral and adaptive because larger items equate generally to more food. There is evidence that other species tend to maximize their food intake by preferentially selecting food of a larger size when foraging (Bern, 1990; Kaufman *et al.*, 1996). Carnivorous lizards (*Varanus albigularis*), for example, showed a bias towards larger snails even if the associated handling cost made them less profitable than the smaller ones (Kaufman *et al.*, 1996). Therefore, food quantity might be a strong motivator in animals' food choice and this should be considered in the context of seed dispersal. The question whether frugivores choose plants that presents bigger amount of fruits when foraging in their natural environment and the consequence for seed dispersal will be investigated in chapter 3.

#### **1.4.4 Why spatial learning might influence seed dispersal**

As described in the section 1.2 and 1.3, two of the most important features of seed dispersal are the dispersal distance achieved from the parental tree and the deposition site (Schupp *et al.*, 2010). For plants that rely on animals as dispersal vectors, these features depend mostly on animals' movement behaviour (Howe and Smallwood, 1982). Ecological studies tend to create a dispersal curves by combining together data on daily animal movement and gut passage time, the so-called dispersal kernel (Wescott *et al.*, 2005). This is a probability density function, which describes the probability of seed deposition at a certain distance from the parental plant (Nathan *et al.*, 2008). In this way, researchers are able to estimate how far away the seeds could be deposited from the parental tree, but without having a clear idea of where the seed is going to be deposited.

Knowing whether and which paths animals are likely to take can help to predict the distance and quality of the deposition sites, allowing more precise estimations of seed shadow and seedling establishment (Wang and Smith, 2002). Whether seed dispersers move at random or follow specific patterns of movement would impact differently on the seed shadow of a tree: random movements, for example, might increase the chances of a frugivore to explore new environments and find new food sources enhancing, as a consequence, the probability that seeds would be transported and deposited in new areas. The seed-shadow created in this case would be unpredictable (Boyer and López-Corona, 2009). Non-random movement, i.e. the animals is able to orientate itself in space and thus can relocate food sources previously used in the environment, would result in a very different seed dispersal pattern: if frugivores frequently visit the same areas and follow the

same paths, the resulting seed shadow would be less spread than the one originated by random movement, creating areas where the density of seeds might be high (Boyer and López-Corona, 2009). For example, Di Fiore and Suarez (2007) found that over eight years, spider monkeys (*Ateles belzebuth*) and woolly monkeys (*Lagothrix poeppigii*) tended to follow repeatedly the same path between fruit trees when foraging. The authors also examined the seed shadow produced by these frugivores and suggested that their movement behaviour limited the dispersal of seeds to specific areas. As a result, seed density would increase in those areas. A similar example is given by Tamarins (*Saguinus fuscicollis weddelli*) that seem to be able to visit food sources in a spatially efficient manner, reducing the distance between foraging points by visiting the feeding sites in progression. The authors suggest that they maintain some form of internal representation of either the specific or relative locations of the fruit trees to one another (Porter and Garber, 2013). If this is the case, the resulting seed shadow of trees would be predictable, and create areas with high seed density along the paths connecting food sources used by tamarins.

Therefore, knowing about animals' spatial learning skills and understanding how these might influence their movements through the environment would help to predict the structure of future plant communities.

#### **1.4.5 Animal spatial learning**

Animals have evolved a multitude of different strategies in order to navigate in the environment. A first simple approach is represented by the use of scent trails. This technique consists in releasing pheromones in the environment

that can be perceived by individuals of the same species (Karlson and Lüscher, 1959; Pearce, 1997). The use of scent trails was well-evaluated among insects (e.g. Calenbuhr and Deneubourg, 1992; Billen and Morgan, 1998; Jarau *et al.*, 2004) and mammals (Skagen *et al.*, 1999; Kozakiewicz and Kozakiewicz, 2004; Porter *et al.*, 2007) and it serves multiple purposes: it could be used to sign the pathway that leads to a food source, to mark territories and to signal the presence of mate or competitors. Typical examples are given by ants, which use chemical signals for marking the route from the food to the nest so that other members of the colony can forage from it (Leuthold, 1975; Beckers *et al.*, 1989; Calenbuhr and Deneubourg, 1992), and rats that use pheromones to relocate food sources in the dark, when other navigation mechanisms are not possible (Lavenex and Schenk, 1998). Moreover, they tend to adjust their spatial preferences according to the presence of the scent of competitors (Wallace *et al.*, 2002a; Roberts *et al.*, 2002). This navigation strategy is proved to be efficient as it successfully allows animals to relocate food sources in the environment without the necessity of learning and memorizing the path or the features of the landscape that leads to them. However, it does not allow flexibility in the movement of the animals, which cannot deviate from the scented path. The seed shadow resulting from seed dispersers that use this mechanism would be confined to specific areas. As a consequence the resulting deposition sites would be characterised by high seed density, which in turn would decrease the survival rate of seeds.

There are other orientation strategies that allow more flexibility in the choice of path to reach the goal. These rely on the spatial learning and memory skills of the animal. The information regarding how to relocate a relevant place can be coded in two different ways: (1) in relation to the animal's position, or

egocentric coding; (2) independent to the animal's position, or exocentric coding (Benhamou *et al.*, 1990). In the first case animals process the path to arrive to the goal location with the information provided by their own movement such as direction and turns. In the second case animals process the information provided by landmarks, i.e. an object(s) or feature(s) in the landscape that it is easily recognizable, and used to individuate the location of the goal (Benhamou *et al.*, 1990).

An example of an egocentric strategy, that does not require the use of environmental landmarks, is defined as dead reckoning or path integration. This navigation strategy allows animals to keep track of its position by taking into account the distance travelled and the changes in direction that have been made (Pearce, 1997; Biegler, 2000; Etienne and Jeffery, 2004). This strategy is usually adopted by animals that live in environments that do not provide visual landmarks, such as deserts (desert ants: Wehner and Srinivasan, 1981; Collet and Collet 2000; Wehner *et al.*, 2006). Path integration has been extensively studied in rats (for a review see Whishaw *et al.*, 2001, Wallace *et al.*, 2002b) and geese (Von Saint Paul, 1982). It seems that path integration is always active and used to compute the position of the animal constantly. The information collected is then used in case the landmarks in the landscape were removed or not possible to be used, e.g. in total darkness (e.g. Von Saint Paul, 1982; Benhamou, 1997; Shettleworth and Sutton, 2005). Path integration allows animals to return successfully to the starting point using also alternative trajectories than the initial one (Wehner and Flatt, 1972; Shettleworth and Sutton, 2005), e.g. returning straight to a starting point after a wandering outward journey (Etienne and Jeffery, 2004). Therefore, it allows more flexibility in reaching a goal, than the use of

scent trails. However, animals that use this strategy are not able to orientate unless they themselves get to a location (Gallistel, 1990). The seed shadows resulting from path integration would be more spread than the one created by scent trail strategy, as animals can undertake new routes to return to the starting point.

Strategies that allow much greater flexibility in the movements are those that involve the use of landmarks, because they allow the animal to orientate from different starting points (Chamizo, 2003). Studies have shown that animals can learn about landmarks present in the environment as they move through it and they are able to build associations between the stimuli and the position of the goal, so that they can eventually use landmarks to navigate towards, for example, a food source (Kelly and Gibson, 2007). If a single feature is located immediately by the goal it is defined as a beacon. Animals learn very quickly to derive the direction of food in respect to this single landmark, without encoding others cues presented in the environment (Robert and Pierce, 1999). The use of a beacon allows the animals flexibility in the route to reach the goal. They are not forced to follow a specific path, but, once they have recognized the visual cues, they can orientate and arrive at the goal no matter the starting point. This kind of spatial learning has been verified in various species of birds (Watanabe, 2005; Flores-Abreu *et al.*, 2012), mammals (Pearce *et al.*, 2001), reptiles (Holtzman *et al.*, 1999; Lopez *et al.*, 2000; 2001) and fish (Odling-Smee and Braithwaite, 2003). Thus, beacon learning seems to be a skill spread among taxa. This might indicate that this navigation strategy is broadly applicable in a variety of environments as long as they contain stable beacons in order to work effectively (Biegler and Morris, 1996). The good flexibility offered by beacon navigation might allow seeds to be dispersed in new areas. However, when animals learned to derive the

fastest direction towards the goal from the cue, they tend to follow this most convenient route (Di Fiore and Suarez, 2007). If this is the case, then the resulting seed shadows would be restricted to specific areas creating deposition sites with high seed density.

When multiple stimuli are provided without a beacon, animals learn to use vectors based on individual cues to define the position of the goal (Chamizo *et al.*, 1985; Collet *et al.*, 1986; Pearce, 1997). If the position of landmarks is changed, studies showed that animals are also able to detect the geometric information of the shape of the experimental arena and use them to define the location of the goal (Cheng, 1986; Margulles and Gallistel, 1988; Sovrano *et al.*, 2002; Pearce *et al.*, 2001). A navigation strategy that implies the learning and use of multiple landmarks is referred as the cognitive map (O'keefe and Nadel, 1978). The cognitive map can be operationally defined as novel route construction that cannot be explained by orienting either by pure path integration or by the use of beacons coincident with the goal (Jacobs and Schenk, 2003). It suggests that animals are able to create a map-like representation of the environment, in which landmarks and places are defined in relation to each other rather than to the body of the animal (Robert and Pierce, 1999). This characteristic would allow animals to navigate flexibly to a goal location from familiar or novel positions with equal capacity (Morris, 1981), and to orient efficiently to every place represented on the map (Pearce, 1997). This allows animals to detour around obstacles (Chapuis and Scardigli, 1993) or take short cuts (Chapuis, 1987). Cognitive maps were identified in mammals (e.g. Chapuis, 1987; Gallistel, 1990; Bovet, 1992), birds (Bingman, 1992; Prior *et al.*, 2002) and, recently, reptiles (chelonians showed map-like behaviour: Salas *et al.*, 2003; Lopez *et al.*, 2000, 2001; Wilkinson, 2007;

2009). This navigation strategy might be advantageous in an environment where cues are multiple and constantly changing, such as rainforests. Studies on primates in the wild are trying to understand whether this is actually the strategy that they use to move efficiently between food sources (Garber, 1989; Di Fiore and Suarez, 2007; Janmaat and Chancellor, 2010; Janmaat *et al.*, 2013). The seed dispersal patterns resulting from the use of cognitive map could be potentially more beneficial to plant dynamics compared to other learning strategies. Thanks to the flexibility and the possibility to make novel short-cuts provided by the cognitive map, animals would have a greater probability of visiting new areas, expanding the seed shadow of plants and increasing their chances to colonize new patches. This would decrease the possibility of high density seed areas being formed, resulting in reduced competition between seedlings.

How frugivores' spatial learning can impact upon seed dispersal will be further discussed in chapter 2.

#### *1.4.5.1 Spatial learning in the red-footed tortoise*

The first study that investigated red-footed tortoise spatial cognition was carried on in 2007 (Wilkinson *et al.*, 2007). A single exemplar of *Chelonoidis carbonaria* was tested in a 8 radial-arm maze, the same type used with rats (Olton and Samuelson, 1976). The red-footed tortoise was found to be able to perform in the maze above chance and the navigation strategy used did not appear to be the result of a stereotyped response strategy, according to which the animal chooses always the next arm of the one previously visited, nor guided by odour cues provided by the food, or scent trails left by the animal. Thus, these first



preliminary results revealed that tortoises are potentially able to learn about spatial location and remember about places recently visited, but the mechanisms underneath this ability were not determined. A proposed hypothesis was that the tortoise was able to use external-maze cues to orientate and navigate efficiently in the maze.

This hypothesis was explored in a subsequent study (Wilkinson *et al.*, 2009), in which the same tortoise was tested in the maze previously described, but this time, in order to control for the external cues, the maze was totally surrounded by a black curtain, so that the subject could not use any of the visual cues offered by the testing room. Attached to the curtain was a set of geometrical shapes that the tortoise could use as cues and whose position could be manipulated by the experimenter. Like in the previous experiment, the tortoise showed an above chance performance in the maze, but this time it adopted a response-based strategy, according to which the subject entered one arm away from the previous arm visited. This strategy was independent of the extra-maze cues provided and surprisingly different from the one emerged in the study of 2007. Once the curtains were removed, so that the tortoise was allowed to use all the visual cues provided by the training-room, results showed that the tortoise had apparently reverted to its strategy as in the study of 2007. This means that the response-based strategy was probably adopted by the tortoise because of the shortage of visual cues provided. These results suggest that the red-footed tortoise is able to use more than one strategy to successfully navigate in the environment, and more importantly, they may be able to adapt the strategy according to what is available in the environment. In 2012 (Mueller-Paul *et al.*, 2012) the same experiment as in 2007 and 2009 was repeated with more subjects, four, in order to

try to generalise the results observed. All of the four tortoises performed above chance, three of them adopting response-based strategy described above with or without the availability of visual cues, and one acting similar to what was observed in 2009, thus changing navigation strategy according to the availability of cues.

The spatial learning skills of the red-footed tortoise were successively tested using a touchscreen (Mueller-Paul *et al.*, 2014). Previous studies involving this apparatus showed that there are similarities between spatial learning performance in the 2D representation of a screen and in a 3D arena (Cheng *et al.*, 2006), thus it is commonly used to investigate mechanisms that regulate spatial learning. Two red-footed tortoises took part in the study. They were initially trained to choose between a right or left stimuli (both identical in appearance) that appeared on the touchscreen. Once the subjects had learned the task, they were tested in an arena where the cues appearance and location replicated those used in the computer simulation. Tortoises chose preferentially the cue on the side that they had learned on the computer screen. This indicated that tortoises were able to transfer the learning from 2D to a 3D environment. Then, the same subjects received a reversal training in the arena. Once they reached the learning threshold, they were tested on the touch screen. Tortoises chose preferentially the side they had been previously trained during the initial touchscreen training and not the one learned during the reversal task in the arena. In this case, tortoises showed the ability to differentiate the information learned according to the specific context. Finally, the subjects were tested once again in the arena, where their side preference was reverted to the one learned during the reversal training phase, confirming that the tortoises were able to differentiate between the context and

apply the information learned to the correct situation. Thus, the results of this study showed that tortoises are potentially able to transfer knowledge in different contexts, and also to differentiate between contexts, retrieving information from the external environment and applying what they specifically learned in it. These findings have important implications translatable to a natural setting. Red-footed tortoises are potentially able to apply the information that they have learned in a specific environment to a new area or a new context, thus navigating efficiently in environments never previously explored, and, at the same time, remain able to retrieve the initial knowledge in case they have to face a situation that they had already encountered, e.g. a visit to an environment that already has been explored.

#### **1.4.6 Why anticipatory behaviour might influence seed dispersal**

Plants, as for all the living organisms, follow a reproductive cycle. They do not produce fruits throughout the whole year, but in specific period/s according to the species and bio-geographical region they inhabit (Momose, 2004). For example, plants in the temperate zone usually follow a seasonal cycle producing fruits exclusively during one season of the year, while some plants species in the tropical zone, can produce fruits multiple times during a year (see Moskovitz and Bjorndal, 1990; Momose, 2004; Chapman *et al.*, 2005). In all environments, plant fruiting cycles are not random but follow specific time intervals over the year (Moskovitz and Bjorndal, 1990; Momose, 2004). This means that a productive and non-productive period of a plant can be potentially predicted and anticipated. It could be hypothesized that being able to learn about plants' fruiting cycle length and to anticipate the next fruiting period, would be an advantage for frugivores.

This would allow them to enhance their foraging activity by avoiding visiting plants without fruits and directing their foraging towards more profitable areas of the landscape. This skill would be particularly helpful, for example, during periods of fruit shortage in the rainforest, such as in months when only 0.2% of plants carry fruits (Chapman *et al.*, 2005; Janmaat *et al.*, 2014). The anticipation of fruiting events would be advantageous also to plants, as they would receive fast seed removal when the fruits are ready to be eaten, decreasing the chances that fruits would be consumed by seed predators or remain under the parental tree. Nectarivores, such as hummingbirds, for example, tend to revisit flowers at the same rate as they re-fill with nectar, suggesting that they learn about the nectar production time interval and synchronise their foraging with it (Henderson *et al.*, 2006). This allows them to save energy otherwise wasted in visiting empty flowers. However, to my knowledge, it is still unclear whether frugivores are actually able to anticipate the time and duration of the presence of ripe fruits on trees.

#### **1.4.7 Do animals perceive the passage of time?**

Animals' ability to perceive the passage of time is a topic that has aroused interest for many years. There are two main approaches for attempting to address the issue: (1) the physiological approach, which deals with the mechanisms that animals evolved in order to perceive environmental cycles and adjust their behaviour accordingly (Dibner *et al.*, 2010), and (2) the cognitive approach, which tries to understand the learning processes behind these behavioural changes and their implications (For a comprehensive review see Roberts, 2002).

Physiological studies have revealed that animals are sensitive of time. Rhythmic processes in organisms from bacteria to human are regulated by self-sustained oscillators, which allow animals to perceive cycles. These are defined as cyclic accumulation of proteins that determine the expression or repression of specific genes that cause changes in the body temperature and/or hormones concentration. (Hardin *et al.*, 1990; Sehgal, 1995; Kondo and Ishiura, 2000; Gachon *et al.*, 2004; McClung, 2006). Among these, the most popular is the circadian clock, which has been found in all the organisms that can perceive light. This mechanism regulates many vital processes, that are based approximately on a 24 h cycle, such as sleep-awake cycles and part of the endocrine system (Pearce, 1997; Roberts, 2002; Gachon *et al.*, 2004). There is evidence that animals are able to perceive rhythms with shorter periods of length, from seconds up to 20 h, called ultradian cycles (e.g, heartbeat frequencies) and longer rhythms, defined as intradian cycles that range from 30 hours to decades (e.g. mating cycles), by using different oscillators (Gachon *et al.*, 2004). However, the fact that animals are sensitive to time does not explicitly mean that they have a concept of time that allows them to view their memories of past events as having occurred at different points over an extended duration (Roberts, 2002). This requires the cognitive approach, which aims to understand how animals' perception of time might be used to anticipate future events, in order to ensure the right adaptive response.

Interval timing, is the perception, estimation and discrimination of durations in the range of seconds-to-minutes-to-hours (Buhusi and Meck, 2005). In order to time these intervals, animals have a pacemaker accumulator (Treisman, 1963) that emits pulses at constant rate and allows to compare between elapsed and remembered intervals of time (Church *et al.*, 1994; Crystal, 2001).

Interval timing has been demonstrated in fish (*Carassius auratus*) (Drew *et al.*, 2005), birds (*Sturnus vulgaris*) (Bateson and Kalcenik, 1997), rodents, in particular in rats (*Rattus norvegicus*) (Crystal, 2001; Buhusi *et al.*, 2002; Gallistel *et al.*, 2004), and primates (*Macaca mulatta*) (Gribova *et al.*, 2002). It is essential, for example, for motor control and speech generation (Buhusi and Meck, 2005). Pavlov was the first to investigate interval timing (Roberts, 2002), giving a dog food at regular intervals of 30 minutes. As response the dog was observed to salivate few minutes prior the food delivery. Similar results were found in pigeons and rats, which learned to press a lever only after a scheduled time prior to food delivery (Crystal, 2001).

As regard circadian cycles, apparently the physiological changes in these oscillators act as retrieval cues that awake animals' memory about, for example, the location of food sources (Roberts, 1998; Roberts, 2002). As a consequence, animals might use circadian cues to anticipate where to find food at a given time of the day (Roberts, 2002). Oystercatchers (*Haematopodidae longirostris*), for example, were observed to visit mud flats at a precise time every day in order to feed on molluscs that are only available for a small window of time (Daan and Koene, 1981); garden warblers (*Sylvia borin*) were able to choose correctly between four chambers that offered food at different times of the day (Biebach *et al.*, 1989); pigeons (*Columbia livia*) were observed to gather in two outdoor lunch areas just before the arrival of people (Wilkie *et al.*, 1996). Therefore, the ability to anticipate where the food is going to be located in the environment would help animals to forage more efficiently, saving time otherwise spent in looking for new food sources. In controlled environments, the standard experimental procedure to examine whether animals can predict a certain event on a circadian cycle is to

provide them with food once a day at a fixed time. Restricting feeding schedules has been shown to restore circadian locomotor activity (Stephan *et al.*, 1979) and create the so called “food anticipatory activity” that is an increase in activity, generally accompanied by an increase in body temperature, in the hour before food delivery (e.g. Stephan *et al.*, 1979; Mistlberger, 1993; Mistlberger, 2009; Waitt *et al.*, 2001; Feillet *et al.*, 2006; Pendergast *et al.*, 2009). Social animals, such as macaques, also showed an increase in aggressiveness prior to food delivery (Waitt *et al.*, 2001), suggesting that the ability to anticipate events in animals that live in groups and/or feed on the same food sources can increase the competition for resources. This might imply that the ability to anticipate food availability is essential when the competition for resources is high, because animals without anticipatory skills would risk finding no food available.

Animals’ ability to anticipate food delivery events will be discussed further in chapter 4, focusing in particular on the advantages that this skill would bring to frugivores and the consequences of such an ability on seed dispersal.

#### **1.4.8 The impact of memory on seed dispersal**

Memory is involved in the storage and recovery of information. Therefore, the study of animal memory could allow us to understand how information acquired at a particular time might influence animal behaviour (Pearce, 1997). Memory can be distinguished into short and long-term, semantic and episodic memory. I will focus here only on the first two, as the cognitive abilities mentioned in the previous sections (discrimination skills, spatial learning and anticipatory events) require these types of memory.

According to Pearce (1997) short-term memory lasts a brief period of time and concerns information about the immediate past, therefore it could help animals to remember which food sources have been recently exploited. Thus, short-term memory could be the incentive that makes animals move away from a particular plant, favouring the dispersal of seeds away from the parental tree, or return to the same place if the food source is not yet depleted. Cognitive studies using the radial arm maze have shown how rats are able to visit each arm of the maze in order to collect food, avoiding arms previously visited on a given trial (Olton and Samuelson, 1976; Olton *et al.*, 1977). This implies that rats are able to remember the spatial location of the arms recently visited, enhancing their efficiency in locating the arms that contain food. Similar results were also found in tortoises (*Chelonoidis carbonaria*) (Wilkinson *et al.*, 2007) and birds (*Manorina melanocephala*) (Sulikowski and Burke, 2007) suggesting that this skill may be adaptive and spread across different taxa.

Long-term memory lasts greater periods of time and is responsible for the duration of information retention (Pearce, 1997). How long the memories are retained varies among species and according to different tasks and consolidation processes (McCaugh, 2000). To give some examples, pigeons (*Columba livia*) proved to be excellent in discriminating among 320 pictures after a two year period (Vaughan and Greene, 1984), scatter-hoarding animals are superb in spatial memory tasks, remembering the location of hundreds of caches after several months (Smulders, 2006; Roth *et al.*, 2012), and territorial sea lions (*Eumetopias jubatus*) remember their rivals over multiple breeding seasons and discriminate between them and novel ‘intruders’ (Gisiner, 1985).



As regards frugivores, to my knowledge, there is a lack of information about whether frugivores' memory of the features of the fruits would affect their future foraging choices, or whether frugivores possess any memory regarding plants fruiting cycles, that might help them to anticipate the presence of ripe fruits. However, there is increasing attention on their spatial memory retention capabilities (Di Fiore and Suarez, 2007; Normand *et al.*, 2009; Noser and Byrne, 2010; Jaanmat and Chancellor, 2010). It might be hypothesised that longer memories about the spatial location of resources are adaptive, because they would enhance the efficiency of animals' foraging, in particular for animals that, like frugivores, rely on food sources that are to some extent predictable: remembering where fruit trees are located would allow them to save energy otherwise spent in the search for fruit. This explains the recent increasing attention on frugivores spatial memory retention capabilities (Di Fiore and Suarez, 2007; Normand *et al.*, 2009; Noser and Byrne, 2010; Jaanmat and Chancellor, 2010; Janmaat *et al.*, 2013). Knowing if a seed disperser regularly returns to an individual fruit tree or consumes fruit from a number of different trees randomly encountered during foraging, would impact very differently on dispersal dynamics of a tree population (Jordano *et al.*, 2007). Boyer (2009) produced a model to investigate this question. The model assumed that foragers knew the environment and retained spatial memory of this environment and, thus, tended to visit the best patches more frequently. Memory-based ranging behaviour generated highly heterogeneous seed deposition patterns, in comparison to a random model, due to the increased probability of seed removal and deposition in the areas more frequented by frugivores. However, improved memory comes with a cost (Mary and Kawecki, 2005), thus spatial memory skills might reflect other necessity of the animals or the seasonality of food availability.

How frugivores' memory skills would impact on their spatial learning, choice of food and anticipatory activity will be tested using a seed dispersal model, in chapter 2, 3 and 4. The results will be further discussed with particular focus on the impact that memory might have on seed dispersal.

## **1.5 Model species**

For the purposes of this thesis, the frugivore red-footed tortoise (*Chelonoidis carbonaria*), was chosen as a model. This species was selected for two main reasons:

1. It is considered an important seed disperser in its ecosystem (Moskovitz and Bjorndal, 1990; Strong and Fragoso, 2006; Jerozolimsky *et al.*, 2009).
2. It is a long-lived animal, thus its foraging choices could have long-term impacts on plant structure in the ecosystem that they inhabit.

The study of the cognitive skills of the red-footed tortoise has recent history and now the cognitive skill of this reptile have never been considered in the evaluation of the species as a seeds disperser. However, studying the cognitive skills of the red-footed tortoise give us important information about how they might locate and choose between food sources and move in the space, information that might be crucial in understanding the quantity and quality of its seed dispersal performance. For example, cognitive studies on spatial learning, revealed that red-footed tortoise is able to navigate in an environment using more than one

technique, being able to adopt a cognitive map-like strategy, learning about visual cues or adopting an response-based strategy to navigate a eight radial arm maze (Wilkinson *et al.*, 2007; 2009). This plasticity allowed the tortoise to reduce the errors in solving the task and be a more efficient forager. More recently, the same species showed to be capable of solving an egocentric spatial task (Mueller-Paul *et al.*, 2014). It can be assumed that tortoises would show the same plasticity in their natural environment. In particular, the maze experiment (Wilkinson *et al.*, 2007; 2009) showed that *C. carbonaria* is able to remember and tend to avoid places in which the food sources have been recently depleted. This is very important in an ecological setting because it means that potentially tortoises would avoid returning to a tree recently visited, and that they would be motivated to move farther from the plant looking for other food sources. This behaviour would increase the chances that the seeds would be deposited far from the parental tree.

However, research focused in understanding the cognitive processes that regulate the foraging activity of red-footed tortoise, and reptiles in general, is lacking. Whether they learn to use visual cues to discriminate between different food qualities and quantities would provide information about how they might select food; investigating their long term memory in spatial and discriminatory tasks would provide important information about how long they will be able to use the information previously learned in order to produce faster and more efficient foraging decisions; no information is available about whether reptiles are able to time and anticipate events, but this is likely to increase the foraging efficiency of the frugivores reptile species that rely on food sources predictable in time. For this reasons, these questions will be investigated in this thesis.

## 1.6 PhD aims and outline

The aim of the present PhD was to investigate the impact of seed dispersers' cognition on seed dispersal, with a particular focus on:

1. *The “where?”: How spatial learning and memory affects frugivores' ability to navigate in the environment and to locate previously visited food sources.*

Understanding the strategies used by animals to move in space will provide important information regarding their movement patterns and, as a consequence, the shape of the seed shadow. This question will be investigated in chapter 2.

2. *The “what?”: How seed dispersers' discriminatory skills and memory affects their choice of fruit, in particular by focusing on their ability to discriminate the quantity and quality of food.*

Understanding food preferences will provide information about which plants in a landscape are likely to attract more frugivores and receive higher seed removal than its neighbours. This question will be investigated in chapter 3.

3. *The “when?”: Does the ability to anticipate events and learn about cycles affect seed dispersal?*

Understanding if seed dispersers are potentially able to predict the fruiting cycle of plants will provide important information about fruit removal rate. This question will be investigated in chapter 4.

Chapter 5 presents a seed dispersal model parameterised specifically with red-footed tortoise ecological and cognitive data. This model aimed to test the impact of frugivores that use forest gaps on seed dispersal. The results of these chapters will be summarised and put in perspective in chapter 6, in which the main findings and the broader implications of the thesis will be further discussed.

# CHAPTER 2

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## 2. WHERE?

### 2.1 Experiment 1. Spatial memory in tortoises: can tortoises remember where to find food?

#### 2.1.1 Introduction

As mentioned in the introduction (section 1.4.5) animals are able to learn about information regarding their own movement and/or features of the environment that can be use as landmarks to orientate. The memory of what they have learned would then further enhance their ability to successfully navigate in the landscape and to relocate relevant resources. Spatial memory has been investigated in a variety of animals (primates: Di Fiore and Suarez, 2007; Normand *et al.*, 2009; Noser and Byrne, 2010; Jaanmat and Chancellor, 2010; Janmaat *et al.*, 2013; elephants: Hart *et al.*, 2008; rodents: Oldon and Samuelson, 1976; Morris, 1984; birds: Clayton and Krebs, 1994; Clayton and Krebs, 1995; Smulders *et al.*, 2010; Flores-Abreu *et al.*, 2012); here I will focus on chelonians as they are the model species for this thesis.

Compared with other cognitive skills, there is a surprising amount of research investigating chelonians spatial memory. Sea turtles, for example, are able to return to specific foraging and/or nesting areas at two to four years intervals (for a review see Lohmann *et al.*, 2008). Turtles were showed to follow remarkably similar paths (Broderick *et al.*, 2007), however, how they relocate specific areas after so much time is still uncertain. It appears that they are able to maintain the direction using a magnetic compass (Lohmann and Lohmann, 1993), which allows them to distinguish among the different magnetic fields corresponding to different oceanic regions (Lohmann *et al.*, 2001), and derive directional information from the movements of the waves (Wyneken *et al.*, 1990; Lohman *et al.*, 1995). Once arrived in proximity to the goal, turtles may use other perceptual cues, such as scent and sounds, to identify with precision the final location (Luschi *et al.*, 2001). Nest site fidelity has also been observed in terrapins (*Chrysemys picta marginata*), who are able to retain memory of a specific location for 6 months to a year, but how they do this remains unknown (Rowe *et al.*, 2005; Mitrus, 2006). In laboratory studies, terrapins (*Clemmys insculpta*) demonstrated to be able to navigate a multiunit maze (Tinklepaugh, 1932). More recently, the terrapin *Pseudemys scripta* was showed to be excellent in navigation based on cue learning, solving a T-maze (López *et al.*, 2000) and a water-maze (López *et al.*, 2001) using multiple or single visual landmarks. The authors suggested that terrapins rely on visual cue to orientate using a cognitive map strategy similar to that postulated for mammals and birds (Wilkinson and Huber, 2012). Gopher tortoises (*Gopherus polyphemus*) are able to relocate the native burrows that they tend to re-use as shelter, in the year after they have hatched (Pike, 2005). The author does not offer any suggestion about the possible

mechanism used, however, tortoises were able to relocate the burrows also after rainy days, which changed the exterior aspect of the nest (Pike, 2005). This suggests that tortoises might have learned about landmarks in the surrounding of the burrows or used a path integration strategy to successfully relocate it. Recent work with red-footed tortoise has shown that they are able to solve an 8 radial arms maze by remembering the arms that they had previously visited - where the food had already been consumed - with or without (using a turn-by-one strategy) the presence of visual cues, suggesting the use of multiple processes to control their navigation (Wilkinson *et al.*, 2007; 2009; Mueller-Paul *et al.*, 2012). The same species was successful in learning an egocentric spatial task, in which the subject had to choose between a left or right option on a touch screen, and performed it correctly after a period of over two months (Mueller-Paul *et al.*, 2014).

According to these findings, spatial memory, i.e. memory responsible for relocate previous location and navigate throughout the environment (Astur *et al.*, 2002), seems to be widely spread among chelonians species, thus it is likely to be adaptive. Corlett (2011) hypothesized that the ability to remember the location of resources in the environment, such as food and water, would be an advantage for the animals, as long as the efficiency gained in foraging overcomes the cost associated to the retaining of information (Mary and Kawecki, 2005). Red-footed tortoise inhabits an environment where plants fruit periodically throughout the year. For example, the family Annonaceae (*Annona sp.*) produce fruits every two months, while plants of the family Sapotaceae (*Ecclinusa sp.*) and Passifloraceae (*Passiflora coccinea*) have a single fruiting period per year, with a gap of eight months in which they do not produce fruit (Moskovitz and Bjorndal, 1990).



Therefore, it would be adaptive for tortoises to be able to retain the information about the location of fruiting plants for prolonged periods, in order to efficiently revisit places where the probability of finding food is higher. Although in the habitat of red-footed tortoises, the Amazonian rainforest, trees of different species produce fruits in different periods of the year (Moskovitz and Bjorndal, 1990), thus food is, to a certain extent, always available, the competition for fruit is extremely high due to the large number of animals (from birds to primates, to small rodents and insects) that rely on them as a primary food source (Fleming, 1979). Thus, the ability to relocate fruiting plants and remember where they are for period of time, that covers at least one fruiting cycle, would be advantageous.

As mentioned above, tortoises are capable of different navigation strategies, that involves or not the use of cues (López *et al.*, 2000; 2001; Wilkinson *et al.*, 2007; 2009; Mueller-Paul *et al.*, 2012). In the general introduction (section 1.4.2) it was mentioned that the choice of navigation strategy could depend on the characteristics of the environment where the animal lives. The South American rainforest is an environment that suffers many changes: for example, the fast growth of opportunistic plants after the rainy season can modify the aspects of stable cues, or tree fall events can open gaps that would change the appearance of large areas in the forest. Thus, an egocentric strategy that doesn't require the use of stable landmarks could be a good choice to orientate in such a changing environment. In Mueller-Paul *et al.* (2014) red-footed tortoises were able to solve an egocentric task, however this consisted of a simple left or right choice. Therefore, it is necessary to further investigate the navigation ability of tortoises without the use of visual cues using a more complex task.

For these reasons, in the experiment to follow, the spatial memory of *C. carbonaria* were tested in an environment, a double Y-shaped maze, where no visual stimuli were provided in order to investigate the ability of tortoises to learn a route that leads to food, relying exclusively on their memory about the path in relation to what learned. After which their spatial long-term memory was tested, to explore whether they were able to remember the direction learned after a time interval of two and eight months.

### **2.1.2 Method**

Fifteen tortoises took part in the experiment: eight of them were adults with carapace length between (17.3-21.4 cm), one medium size (carapace length= 12.8 cm) and six juveniles with carapace length that ranges between 7.7 and 9.3 cm. Two of the adult tortoises were experimentally naïve, while all the other subjects had taken part in other cognitive studies (e.g. spatial cognition (Wilkinson *et al.*, 2007; 2009; Mueller-Paul *et al.*, 2014), social cognition (Wilkinson *et al.*, 2010a,b), visual cognition (Wilkinson *et al.*, 2013)). When the tortoises were not taking part in the experiment, they were housed in three different pens according to their size. The adults were housed in a big open top enclosure of 4x3 m. The medium size tortoise was housed in an enclosure of 1 x 1.5m together with another medium size tortoise that didn't take part in the experiment. The juveniles were housed all together also in an enclosure of 1 x1.5m. Each enclosure contained water ad libitum, shelters, hot spot with heat and UV lamps. The lights were kept on a 12 hour cycle (from 7am to 7pm); the soil was covered with bark that was damped once a day. The temperature of the room

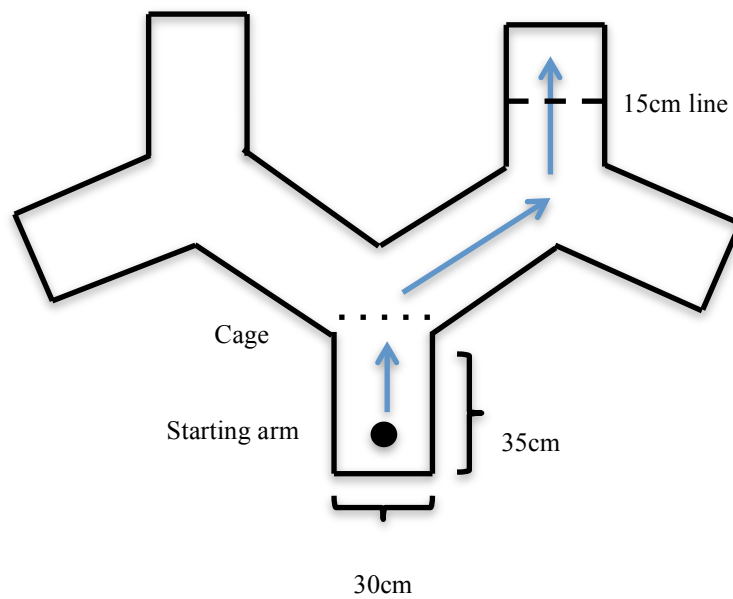
was kept between 27 and 30 degree. Tortoises were not food deprived but were fed after the completion of the daily training, with a variety of fresh fruits and vegetables.

#### *2.1.2.1 Apparatus*

The maze was a double Y-shaped maze (fig 2.1). All the arms of the maze had the same dimensions (30cm width, 35cm length). The internal part of the maze was completely covered by a yellow plastic in order to remove any possible visual cues and ensure ease of cleaning. All the arms therefore looked identical to each other. Yellow was chosen, because it was different from the colour of the walls of the enclosures where the tortoises were usually housed and also different from any other maze in which the tortoises has previously been tested.

#### *2.1.2.2 Habituation phase*

Tortoises were individually introduced into the maze for a period of 20 minutes twice a day for two consecutive days. During this time, they were free to explore the maze with no restrictions. On the third day 8 pieces of food were scattered throughout the arena. This was continued until the tortoise ate at least 5 pieces of food. Upon reaching this criterion the tortoise was considered habituated and ready to start the training phase. The maze was cleaned between habituation trials, in order to remove any scent trace left by other tortoises. All the fifteen subjects ate the food in the maze on the third habituation day, thus they all rapidly proceeded to the training phase.



**Figure 2. 1 Representation of the maze used in the long- term memory experiment. The black point indicates the starting point. Blue arrows indicate one of the four possible paths.**

### *2.1.2.3 Training phase*

Four different paths were possible in the maze; in order to eliminate possible bias, the goal direction assigned to the tortoises was counterbalanced across individual. Four subjects were randomly assigned to the directions left-left, right-right, left-right and three subjects to the direction right-left. At the beginning of a trial a tortoise was placed in the starting arm (fig 2.1) enclosed by a cage, so that the tortoises could see through but could not move around the maze. Once they had looked at both sides, the cage was removed and the trial started. Tortoises were given two minutes (from when they started walking) to solve the task and reach the correct arm. A choice was considered made when both front legs of the tortoise passed a line (indicated on the outside of the maze) 15cm inside each arm. If the tortoise reached the correct location, the trial ended and the

tortoise was rewarded with a small piece of favoured food (e.g. strawberry, dandelion, mango) dropped inside the goal arm after the choice was made. If the choice was incorrect, i.e. the tortoise entered in one of the three wrong arms, the tortoise was removed from the arena without any reward. If the tortoise did not start moving within three minutes of being released then it was removed from the arena and the trial was repeated later. If the subject did not complete the task in the two minutes allowed, it was removed from the maze and the trial was repeated later. The experimenter was in the room for the duration of the experiment, and was positioned behind the starting point of the maze thus, I could not be seen by the tortoises while they were performing the task. The maze was cleaned at the end of each trial in order to remove any possible scent cues. In between the trials, the maze was rotated in different directions (chosen at random), so that tortoises could not use extra-maze cues from the test room to orientate. A session was composed by 15 trials. When a subject reached the learning threshold of 12 correct trials over the last 15 trials (80%) three times in the last five training sessions, the task was considered learned. If an individual did not reach the criterion after completing 250 trials it was excluded from the experiment. This maximum threshold corresponded to five times the number of trials needed to learn the task of the best performer among the subjects - 50 trials.

#### *2.1.2.4 Two months retention test*

After reaching the learning threshold, the tortoises were not exposed to the apparatus for a period of two months. After this period of time the tortoises were given six consecutive trials, in which they were asked to complete exactly the

same task as during training except that the tortoises did not receive differential feedback.

#### *2.1.2.5 Re-training*

Once the test was completed, tortoises were re-trained on the same direction on which they were initially trained and tested. The procedure followed and the learning criteria were exactly the same as in the initial training.

#### *2.1.2.6 Eight months retention test*

This test was identical to the first test, except that it took place eight months after retraining. A total of nine subjects were tested; one had to be excluded due to a health issue.

#### *2.1.2.7 Data analysis*

The analysis was performed with the software R, version 3.0.2. Data from the test trials were analysed on an individual level using a binomial test, in which the number of correct trials were compared to chance (0.25). Also for the group analysis a binomial test was performed, this time entering the number of individual that performed above chance as success, and the total number of subjects tested as total events. Moreover, the number of trials needed to reach the

learning criterion in the initial and re-training were compared using a Wilcoxon-paired test.

### 2.1.3 Results

#### 2.1.3.1 Training

Three tortoises were excluded in the early stage of the training because they constantly refused to move; two of them were far from reaching the learning criterion after 250 trials, thus were excluded. A total of ten tortoises, from the initial fifteen, successfully completed the training and proceeded to the test phase. Individuals varied in how quickly they learned the task (table 2); the average number of trials needed to reach the learning criterion was 129.8 (SD  $\pm$  55.27; range 50-215 trials).

**Table 2 Individual data of trainings and tests**

Tortoise	First training	1 <sup>st</sup> Test	Binomial test	Second training	2 <sup>nd</sup> Test	Binomial test
Anton	130	<b>3</b>	$p = 0.17$	53	<b>5</b>	$p < 0.01$
Margot	155	<b>5</b>	$p < 0.01$	45	1	$p = 1$
Otis	95	2	$p = 0.64$	96	0	$p = 0.34$
Timothy	120	2	$p = 0.64$	236	0	$p = 0.34$
Esme	114	<b>4</b>	$p = 0.037$	45	3	$p = 0.17$
Aldos	60	0	$p = 0.34$	201	<b>1</b>	$p = 1$
Betty	149	<b>4</b>	$p = 0.037$	70	/	/
Darwin	215	<b>4</b>	$p = 0.037$	59	<b>4</b>	$p = 0.037$
Savina	50	<b>4</b>	$p = 0.037$	45	<b>3</b>	$p = 0.17$
T19	210	2	$p = 0.064$	76	2	$p = 0.64$

**Table 2. Number of trials to reach the learning criterion in the initial (first training) and re-training (second training) and number of correct trials performed in the tests after 2 months (1<sup>st</sup> test) and 8 months retention time interval (2<sup>nd</sup> test), with corresponding binomial tests for each tortoise. The numbers in bold in the test one and two indicate the subjects that performed correctly the first test trial. The ‘/’ indicates that the tortoise did not participate to the test.**

#### *2.1.3.2 Two months retention test*

After two months of no interactions with the maze the binomial test revealed that five tortoises performed above the expected chance in the test (binomial test:  $p = 0.03$ ). However, at a group level, the performance of the tortoises was not different than what would be expected by chance (binomial test:  $p = 0.13$ ), suggesting that tortoises were not able to remember the direction to follow in the maze. However, this result could be caused to the procedure of the test itself: tortoises in fact were not rewarded even if they were making the correct choice. Although this is necessary - during the test the animals should not be reinforced, because then their memory would not be tested - it could discourage the animal toward the correct choice. Thus, the data concerning only the first trial of the test were analysed, as its outcome is not affected by the absence of the reward, entering in the binomial test the number of tortoises that performed correctly in first test trial as “success” (table 2). In this case, results revealed that tortoises performed above chance at group level (binomial test:  $p = 0.019$ ) (fig. 2.2), suggesting that they were able to remember and choose the correct path in the maze after two months retention time. The performance in the test was not correlated to the number of trials that tortoises needed to learn the task ( $r = 0.09$ ,  $df = 8$ ,  $p = 0.38$ ).

#### *2.1.3.3 Re-training*

Once again, tortoises varied in how quickly they re-learned the task (table 2). The average number of trials used in the re-training in order to reach the learning criterion was 92.6 ( $SD \pm 68.80$ , range 45-236). A Wilcoxon test revealed



no significant difference in tortoises' performance during the initial training and the re-training (Wilcoxon test:  $p= 0.32$ ). However, considering only the tortoises that performed correctly in the first test trial, results show that they were able to reach the learning criterion significantly faster than in the initial training (Wilcoxon test:  $p< 0.031$ ). A correlation was found between the number of correct trials performed in the test and the total number of trials employed in the re-training ( $r= 0.58$ ,  $df= 8$ ,  $p= 0.01$ ), therefore the better the tortoise performed in the test, the smaller number of trials they needed to reach the learning criterion in the re-training (fig 2.3).

#### *2.1.3.4.Eight months retention test*

9 subjects were tested after 8 months retention time. At individual level, two tortoises performed above chance in the test (table 2)(binomial test:  $p< 0.01$  and  $p= 0.037$ ). At a group level, a total of four subjects performed correctly the first test trial, and the binomial test revealed that their performance was not different from what expected by chance ( $p= 0.24$ ) (fig 2.2), suggesting that tortoises failed in remembering the task after eight months in which they were not exposed to the maze.

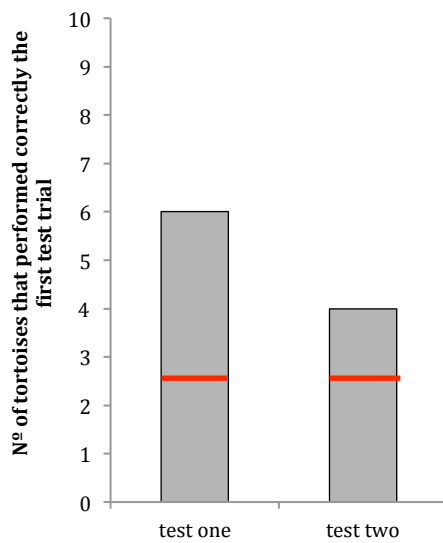


Figure 2.2 Number of tortoises that performed correctly in the first test trial in the two tests (test one= 2 months retention interval; test two= 8 months retention interval). The red line indicates the chance levels, 2.5 in test one (n subjects=10) and in test two (n subjects =9).

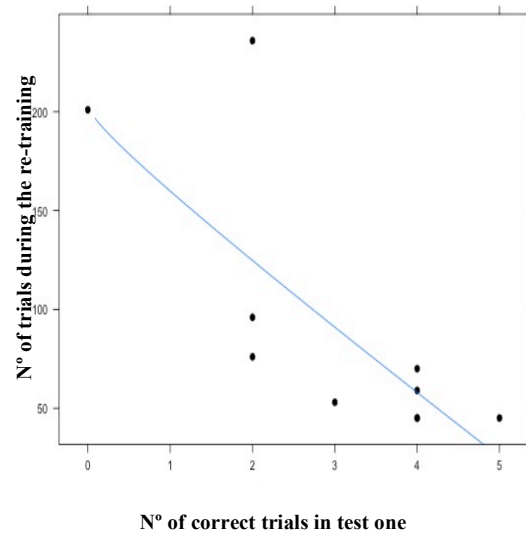


Figure 2.3 The number of correct trials performed in test one (2 months retention interval) and the number of trials needed to reach the learning criterion during the re-training.

#### 2.1.4 Discussion

The results suggest that tortoises are able to learn a path without the help of any visual cues, remembering the sequence of turns required to get to the reward. Thus, tortoises were able to orientate and navigate the maze using an egocentric strategy, according to which the subject functions as its own central point of reference, and the animals remember the sequence of motor movement necessary to reach the goal, e.g. turn left, turn left (Carrillo-Mora *et al.*, 2009; Rosati *et al.*, 2014). Moreover, red-footed tortoises were able to remember the path learned after a retention time interval of two months. The results support the findings of Mueller-Paul *et al.* (2014) where tortoises successfully learned an egocentric spatial task and remembered it for over two months. This means that in their natural environment red-footed tortoise would be able to relocate trees for all

the length of the fruiting period, which is usually between two and three months (Moskovitz and Bjørndal, 1990), but also between fruiting periods. This ability would help tortoises to visit more frequently areas in which the probability of finding food is higher. This is in line with the hypothesis of Corlett (2011), who suggested that the most efficient foraging strategy of frugivores would be to return to the location recently visited for the duration of tropical fruit crop. Tortoises' performances in the test were not related to the amount of trials needed to learn the task, suggesting that the individual learning skills did not have any kind of influence on their memory. It is interesting to notice that the two subjects that needed the greater number of trials to reach the learning criterion (Darwin and T19) were naïve to cognitive experiments, suggesting that previous experience counts. This implies that, in a natural scenario, young tortoises would be less efficient than older ones in relocating food or navigating around the environment, because they have to build their own experience. Tortoises that performed above chance in the test at the individual level were able to re-learn the task faster than in the initial training, providing further evidence that they actually remembered the location of the goal arm. However, tortoises failed in remembering the correct path after a retention time interval of eight months. The poorer performance in the second test could be due to the navigation strategy they were "forced" to use to solve the task. Tortoises are highly visual, with the ability to discriminate shapes and colours in stationary stimuli (Burghardt, 1977; Arnold and Neumeier, 1987). Therefore, it is plausible that if they had been allowed to use visual cues to orientate, they would have retained the spatial information for longer time. For example, Davis and Burghardt (2012) found that terrapins were able to remember a visual task for up to two years. In the experiment that will be

presented in the next chapter, red-footed tortoises proved to excel in remembering an association learned after a period of 18 months. Thus, it could be suggested that for longer retention time intervals, tortoises need some additional cues in order to successfully remember a location.

Further study is needed to understand the mechanisms tortoises rely on to use spatial information in their natural habitat, bearing in mind that foraging on spatially fixed food sources could have influenced the evolution of their spatial memory skills. Studies on primates often suggest that the evolution of cognitive skills is due to their complex social interactions (de Waal 1982; Byrne and Whiten 1988). However, a recent study on lemurs (Rosati *et al.*, 2014) found that the most frugivorous species tested presented the most accurate spatial memory, in particular comparison with folivores species. Tortoises are solitary animals, although they can learn from conspecifics (Wilkinson *et al.*, 2010b), their learning skills depend mostly on their own experience. Therefore, ecological factors may be important predictors of animal cognition and should be incorporated in cognitive studies in order to understand the evolution of particular cognitive mechanisms.

### **2.1.5 Implication for seed dispersal**

The results of the spatial memory experiment have important implications for seed dispersal: the fact that they were able to remember the location of food for a period of two months, which is equivalent to the duration of the production of fruit of some tropical plants (Moskovitz and Bjorndal, 1990), might suggest that tortoises have the capacity to re-visit food sources previously encountered

instead of looking always for new ones (Lohmann *et al.*, 2008). This would lead to an increase in their foraging efficiency, as showed in the study of Di Fiore and Suarez, 2007. As a consequence, spatial memory skills would increase the chances that seeds would be eaten and dispersed by a legitimate seed disperser, decreasing the possibility that seeds would be eaten by seed predators, or simply left under the parental plant where the mortality may be higher (Schupp *et al.*, 2010). Moreover, it could be hypothesized that if plants produce fruit at smaller time intervals (e.g. every one-two months), it could probably invest less in attractiveness because it can rely on the memory of the dispersal vectors, however, to my knowledge, whether this correlation exists, has not yet been investigated. In contrast, plants that produce fruits only once a year should invest more resources in producing signals, such as bright coloured fruits with strong scent, because after such a long period of time it is more likely that animals would forgot about their location. Moreover, in the case that frugivores would return to the plant at regular intervals, they wouldn't find food. This could extinguish their behaviour, making the animal lose interest in that specific tree. Therefore, it would be interesting look at plant signals in relation to their fruiting time and the memory of the principal disperser.

## **2.5 Creating prediction: introducing spatial memory in seed dispersal model**

### **2.5.1 Why use a model?**

Models simulate aspects of the real world and are used as instruments to test and explore the implications of hypotheses, or exploring preliminary ideas, with the aim to support or disprove them (Weiner and Conte, 1981). They are used mainly when empirical studies are not possible, time consuming and demand considerable human resources, or to make predictions about the evolution of an environment in a long-term scale. For these reasons, models have recently been employed in the study of seed dispersal and their use is constantly increasing: field data on seed dispersal are essential but very scarce and approximate, in particular concerning long dispersal distance (Cain *et al.*, 2000). Models provide partial solution to this problem allowing to test and to predict the potential outcome of seed dispersal events on a long-term scale. Genetic methods provide precise information about the movement and establishment of seeds using genetic markers (Nathan *et al.*, 2003). However, this method provides a good estimation of effective dispersal, considering only the seeds that are successfully established (Cain *et al.*, 2000), but without giving information about the dispersal mechanism per se, e.g. which factors influence the shape of the seed shadow. Moreover, it requires the collection of numerous samples and extensive and expensive laboratory work (Nathan *et al.*, 2003). Another method, which achieves the similar results to the genetic one, but that is broadly applicable, is the use of biological markers, such as stable isotope, that can be sprayed on the plants during

their flowering stage (Carlo *et al.*, 2009). The advantage of this method is that it is faster and cheaper than the genetic one, but it allows to follow up a maximum of three enriched plants, therefore it cannot be employed in studies that want to measure the dispersal of many individuals from the same site (Carlo *et al.*, 2009). As for the genetic method, using biological marker is useful to quantify the microhabitats where seeds are deposited and grow but it does not provide any information about the journey of the seeds. Thus, the two most precise methods to estimate seed dispersal effectiveness do not allow predicting how the seed shadow would suffer in case of the removal or changes of the seed dispersal agent, e.g. animals or wind. In order to determine the flexibility and to fully understand the seed dispersal process, it is necessary to know and predict how the factors that affect it behave in the ecosystem. This is one of the reasons why mechanistic models are a very useful in this discipline, as they aim to explain the probability distributions of seeds, instead of only the density of the seeds deposited in relation to the distance as in the empirical models (Bullock and Clarke, 2000). Thus, potentially they could give better insights into the dynamic of plant populations.

### **2.5.2 Mechanistic model for seed dispersal by wind**

Models of seed dispersal by wind have proven to be reliable, providing progress in understanding and predicting the outcomes of this mechanism (Greene and Johnson 1989, Bullock and Clarke 2000, Nathan *et al.*, 2001). This assessed reliability is due to the fact that simulations of wind dispersal are parameterised with information on abiotic factors, such as weather condition, wind speeds, seed departure height, which follow physical laws, thus, are easily predictable. An

example is given by Nathan *et al* (2001), who developed a model which incorporates the full natural variation of the factors that play a role in wind dispersal. They validated their model with field data collected using seed traps and found the prediction of the model accounted for the 83 to 90% of variation observed in the data set. A second example is represented by a model on secondary dispersal by wind that considered the traits of seeds, wind conditions and obstacles to seed movement, and explained between the 70-77% of dispersal over 25m (Schurr *et al.*, 2005). Therefore, by knowing accurately the habitat traits, seeds characteristics and main wind condition of a particular environment, is it now possible to achieve good approximation of the seed shadow of trees that use wind, or other abiotic factors, as a dispersal vector. Thus, a reliable model is a powerful instrument, not only because it allows saving years of data collection in the field, but also because it overcomes the context-specificity of the empirical data (Cousens *et al.*, 2010) and offers the possibility to accurately simulate what would happen if the variables that affect seed dispersal, e.g. wind strength, are suffering changes, or to test how the environment would react in extreme conditions.

### **2.5.3 Model for seed dispersal by animals**

Mechanistic models of endozoochory are far more challenging than those that model abiotic dispersal. Having animals as dispersal vectors adds degrees of complexity primarily due to the fact that animal decision-making and movements are not easily predictable and difficult to quantify (Russo *et al.*, 2006). Multiple factors impact on animals' behaviour, decisions and movement, such as predation



risk, distribution of resources (food, water and shelter), the presence of mating partners, intra and inter-specific interactions (Boinski and Garber 2000, Westcott and Graham 2000, Russo *et al.*, 2006), but also the cognitive skills of the animal, all of which can differ radically from one species to another. As a result, despite the numerous researches on this topic, the ability to predict dispersal pattern by animals remains undefined (Cousens *et al.*, 2010). The common method to simulate animals movements is usually represented by constrained random walks (Turchin, 1998; D'hondt, 2012), which means that the animal moves at random in the environment but preferring some part of the landscape over other, e.g. areas with food or favourite vegetation type. Thus, this preference drives movement toward what is perceived as the better part of the landscape. Despite constrained random walks representing a good approximation of what an animal might do, they are far from being accurate. Knowing precisely how animals use the environment and how they move around it would help to predict the position and quality of the deposition sites, allowing more precise estimations of seedling establishment (Wang and Smith, 2002). To date models of endozoochory have taken into account how the distribution of food or other resources (Aparicio *et al.*, 2013), behavioural traits of the animal, such as use of home ranges (Holbrook *et al.*, 2002), resting sites (Russo *et al.*, 2006), lek sites, diurnal habits (Westcott *et al.*, 2005) and social interactions (Beecham and Farnsworth, 1998) impact on the use of space by animals. From these studies, it has been recognised that non-random movements driven by behavioural habits (Beecham and Farnsworth, 1998) influence dispersal curves and deposition patterns (Schupp *et al.*, 2002). For example, the consideration of seed dispersers' resting sites or use of latrines, led to the prediction that areas with high seed density, therefore with high

seedling mortality, can occur also far from the parental trees (Russo and Augspurger, 2004; Russo *et al.*, 2006), or plants in aggregation suffer a better seed removal than isolated ones (Carlo and Morales, 2008). A step that could bring these studies further would be to investigate what determines the behaviour of the animals, instead of limiting the simulation to the simple behaviour of the animal in a case of study (Cousens *et al.*, 2010). As explained in the first section of this chapter, cognition could be the key that would bring a deeper understanding how animals impact on seed dispersal. To date only a couple of studies in the literature attempted in using cognition to model animal movement (Boyer and Walsh, 2010; Avgar *et al.*, 2013), showing that spatial memory can affect the trajectory of the animal around the environment, resulting in non-random movement. However, they did not extend the concept to investigate the consequences of memory on seed dispersal.

For this reason I introduced animal cognition in seed dispersal model. In particular, in this chapter, I focused on determine how different spatial memory skills would affect quantitative and qualitative the seed shadows of trees.

#### **2.5.4 Method**

This model is based on the perception and memory-based movement model described by Avgar *et al.* (2013) and the seed dispersal model of D'hondt and Hoffmann (2011).

#### 2.5.5.1 *The animal*

Consider an animal characterised by a single parameter, state  $s$  ( $0 \leq s \leq 1$ ), which equate to hunger; when  $s$  is low the animal is considered to be relatively hungry, and when  $s$  is high the animal is relatively sated. During each of  $T$  discrete time steps  $t$  ( $t = 1, 2, \dots, T$ ) this animal moves stochastically around a landscape in state-dependent manner, during which it ingests, defecates and disperses seeds.

#### 2.5.5.2 *Perception, memory and assessment of habitat quality*

The animal moves around stochastically in a landscape,  $A$ , modelled as a heterogeneous hexagonal grid of cells,  $i$  (Avgar *et al.*, 2013). Each cell is characterised by its spatial coordinates and the values of two habitat components, the background landscape ( $A_{i,1}$ ) and the vegetative landscape ( $A_{i,2}$ ), each taking values in the range  $[0,1]$ . Higher values are more attractive to the animal (for example indicating more food, preference for landscape features such as shelter) and lower values less attractive (for instance, less food, un-preferred habitat type). The animal is assumed to assess the quality of surrounding cells using sensory information, which attenuates with distance (perception) and accumulates over time (memory). At time step  $t$  the animal's perception,  $p$  ( $0 \leq p \leq 1$ ), of cell  $i$  for a given layer  $j$  of the landscape is

$$p_{i,j,t} = A_{i,j} e^{-\alpha d_i}, \quad (1)$$

where  $d$  is the (hexagonal) Manhattan distance from the animal's current location to cell  $i$ , and  $\alpha$  ( $\alpha > 0$ ) is the sensory attenuation coefficient. The term  $e^{-\alpha d_i}$  therefore describes the proportion of information perceived at distance  $d$ ; when  $d = 0$  (i.e., cell  $i$  is occupied by the animal) the animal has perfect perception, with the proportion of information perceived declining exponentially with increasing  $d$ . This sensory information is subsequently committed to the animal's memory,  $m$  ( $0 \leq m \leq 1$ ), which decays with time such that

$$m_{i,j,t} = p_{i,j,t} + (1 - e^{-\alpha d_i})(m_{i,j,t-1} e^{-\beta}), \quad (2)$$

where  $\beta$  ( $\beta \geq 0$ ) is the memory decay coefficient, which models the proportion of information retained in the memory. When  $\beta = \infty$ , decay is instantaneous and so the animal has no memory other than what can be currently perceived; when  $\beta = 0$ , there is no decay and the animal retains in its memory all previously perceived information; when  $0 < \beta < \infty$  memory decays exponentially over time.

The subjective quality  $q$  ( $0 \leq q \leq 1$ ) of different locations in the landscape is a function of the perceived and/or memorised habitat characteristics, the animal's current state and its travelling propensity, modelled here as  $e^{-\gamma d_i}$ , where the 'friction' coefficient  $\gamma$  ( $\gamma \geq 0$ ) (*sensu* Avgar *et al.*, 2013) models how far the animal is willing or able to travel by reducing the attractiveness of all landscape features as a function of distance. State affects the relative importance given to

each landscape layer, such that for low values of  $s$  higher relative importance is given to the vegetation (food-containing) layer, and when  $s$  is high greater relative importance is given to the background layer. The subjective quality of a cell is therefore given by

$$q_{i,t} = \left( m_{i,1,t}s_t + m_{i,2,t}(1 - s_t) \right) e^{-rd_i}. \quad (3)$$

When applied to all cells, the resulting map is the subjective landscape; the landscape as it is viewed from the perspective of the animal at a particular point in space and time (Avgar *et al.*, 2013).

#### 2.5.5.3 Movement

At each time step the animal can choose to either remain in its current cell, or move to one of the six (equidistant) adjacent cells. Specifically, animal movement behaviour was modelled as a series of discrete probabilistic decisions, based on attraction to specific cells within the animal's subjective landscape: the preference for remaining in the current cell,  $k$ , is given by  $q_{k,t}$ ; the preference for moving to a particular adjacent cell is given as the maximum value of all cells in the subjective landscape within a  $60^\circ$  cone centred on the direction of that cell. To ensure a correlated random walk in relatively homogeneous landscapes, these preferences are then multiplied by the probability of moving in a given direction drawn from a von Mises distribution with a mean direction ( $\mu_m$ ), which is equal

to the current direction of travel, and concentration parameter  $\kappa_m$ . These combined preferences are then re-scaled so they sum to unity, giving the probability of moving (or otherwise) to a given cell.

#### 2.5.5.4 Feeding, gut passage and seed dispersal

Having entered a cell, the animal will feed if that cell contains vegetation. The amount of food consumed,  $f$ , is proportional to the value of the vegetative layer of the occupied cell,  $k$ , scaled by the state of the animal at the previous time step (such that relatively hungry animals will eat proportionally more than relatively sated animals)

$$f_t = A_{k,2}(1 - s_{t-1}). \quad (4)$$

It is assumed that all ingested food contains seeds, and that these are added to the animal's 'gut matrix',  $\mathbf{G} = [g_t]$  (*sensu* D'hondt and Hoffmann 2011), where  $g_t$  holds information on the amount of seeds ingested at each time step. For simplicity, it is assumed that the proportion of seeds ingested, and their probability of being destroyed through destruction and/or digestion, is fixed; the number of viable seeds available for excretion when the animal defecates is therefore directly proportional to the total amount of food eaten (i.e.  $f_t \sim g_t$ ).

At each time step, the animal defecates with a probability drawn from a cumulative lognormal probability density function, defined by  $\mu_d$  and  $\sigma_d$ , such

that the probability of defecation increases with time since the last defecation event (D'hondt and Hoffmann 2011). Such a distribution has been shown to provide good fits to empirical gut passage data (e.g. Rawsthorne *et al.*, 2009; D'hondt and Hoffmann 2011). If there are seeds present in the gut at the time of defecation, a proportion of these are excreted. This proportion is determined by the time that has passed since their ingestion, modelled as the cumulative lognormal probability density function defined by  $\mu_s$  and  $\sigma_s$ .

Food consumption also affects the animal's state. State decreases over time as a function of the hunger coefficient  $\lambda$  ( $0 \leq \lambda \leq 1$ ), which encapsulates the non-linear relationship between food intake and hunger, and increases as a function of food consumed, scaled by the satiation coefficient  $\kappa$  ( $0 \leq \kappa \leq 1$ ), such that

$$s_t = \lambda s_{t-1} + \kappa f_t. \quad (5)$$

#### 2.5.5.5 Parameterising the model

A 1600 cell landscape grid, characterised by randomly generated patchy habitat quality, ranging in value between 0 and 1. The vegetative layer contained 2 randomly placed plants, each occupying a single cell with a value of 1 (maximum attraction). One of the plants is considered the “focal plant” which the animal knows, because its location in the grid coincides with the starting point of the animal, while the other, the unknown plant, is located at a random distance from the focal tree.

The state of the animal is initially set to 0.5 (alpha is fixed at 0.5). The value of the parameter  $\beta$  was varied at random using the matlab function `randel(0.99,1)` where ( $\beta = \infty$ ) indicates no memory and ( $\beta = 0$ ) complete memory. Animal memory is initially set to 0 (i.e., when  $t = 1$ ,  $m_{i,j,t-1} = 0$  in eq. 2). The animal is introduced in the landscape in the same hexagon of one of the two plants, selected at random. This starting position was chosen, otherwise substantial part of the simulation was employed initially by the animal to locate the plants in the landscape. Animals were assumed to have died when their state dropped below a threshold (set at 0.001). The model was run over 10,000 time steps and replicated 1000 times.

**Table 3 Overview of model parameters.**

Parameter	Values explored
$\alpha$ : sensory attenuation coefficient	0.5
$\beta$ : memory decay coefficient	Drawn from an exponential logarithm distribution (0.99,1)
$\gamma$ : friction coefficient	0.07
$\kappa$ : satiation coefficient	0.1
$\lambda$ : hunger coefficient	0.99
$\mu_d$ and $\sigma_d$ : parameters of the cumulative lognormal probability density function describing the probability of defecation	4; 0.2
$\mu_s$ and $\sigma_s$ : parameters of the cumulative lognormal probability density function describing the probability of seed excretion	1; 0.2
$T$	10,000
$\kappa_m$ : concentration parameter for von Mises distribution	1.83
<i>Death threshold</i>	0.001



<i>Plant Quantity</i> : value of the plant as it appears in the vegetation map	1
<i>Plant Quality</i> : quality coefficient of the plant	1

#### *2.5.5.6.Data analysis*

My interest was in analysing the impact that animal memory can have on the seed dispersal process, thus, the quantitative and qualitative aspects of seed dispersal (see section 1.1.1 of chapter 1) were explored. For the quantitative part the total amount of seeds dispersed in the environment and the amount of seeds dispersed from each plant were analysed. As regarding the qualitative aspects, the long dispersal distance of seeds from the parental tree was studied. The analysis of the data was performed by multiple regression creating a linear model with the function `lm` in R 2.15.0 (Crawley, 2005). A model complete with all the explanatory variables (main effects and interactions) that needed to be tested was created. Then, the minimal adequate model was obtained by reducing the full model to a point where all the remaining predictors were significant. This was done one predictor at time by comparing with likelihood ratio tests, the model to a reduced model lacking the term of interest (Crawley, 2005). The same procedure was used to test different interactions and main effects in the simulations to follow in the next chapters.

Animal movement and animal death rate were examined to understand whether memory would benefit frugivores in terms more efficient travelling around the landscape and survival. The procedure described above was used to analyse data on animal movement, in order to examine how memory impacts on

the way that animals explore the landscape. The analysis of data concerning the death rate of animals with different memory followed a different procedure. Since in the simulation some animals died before reaching 10000 timesteps, while in other iterations the animal survived for all the 10000 timestep, and it is unknown when they would perish, an exponential survival model with a censoring indicator (=status) was used for this purpose. To perform the analysis, a value of 1 was assigned to the animals that died before the 10000 timesteps to indicate that the response was a time at death. A value of 0 was given to the individual that completed the 10000 time steps, thus still alive at the end of the simulation. To perform the analysis the function `survreg` in the package `survival` of R 2.15.0 was used (Crawley, 2007). In the null model the time of death with the respective status was entered as dependent variable, memory of the animal and plant distance were entered as fixed terms. The distribution was specified as “exponential”. The procedure to test the significance of the interactions and main effects, followed the same steps as above: a second model lacking the term of interest was created and compared with the null model using likelihood ratio tests. Once the model that best described the data was found (all the included terms/ covariates were significant), the same model was fitted with other distribution (extreme, gaussian, logistic). Once again, these models were compared with ANOVA and the resulting one with the smallest residual variance was chosen as the best fit for the data (Crawley, 2007).

### 2.5.5 Hypothesis tested

The model aims to test the impact of spatial memory on seed dispersal and how this might interact with the distance between the plants. In this section, simulations of two different landscapes are compared. These were parameterized in exactly the same way (see the method) with the only difference that in one model the availability of food in the landscape was unlimited, thus no matter how many times the animal fed on a plant, the food was never depleted or decreased (the value of the plant was fixed at the maximum value, 1, see method). The second model, simulated an environment where the availability of food was limited, thus where the food could be depleted; every time an animal fed on a plant, the value of that plant was decreased by the amount eaten, until complete depletion (value of the plant reaches 0, see method).

The following hypothesis were tested:

H<sub>1</sub>: The memory of the frugivore predicts the total amount of seeds dispersed in the environment.

H<sub>2</sub>: The interaction between memory and the distance among the plants predicts the total amount of seeds dispersed in the landscape.

H<sub>3</sub>: The interaction between memory and the distance among the plants explains the amount of seeds dispersed by each plant.

H<sub>4</sub>: The interaction between memory and the distance among the plants predicts the long distance dispersal of the individual plant.

H<sub>5</sub>: The interaction between memory and the distance among the plants explains the proportion of landscape travelled by the animals.

H<sub>6</sub>: Memory predicts the time of death of the animals.

The predictions are that (1) animals with higher spatial memory (slow memory decay,  $\beta \rightarrow 0$ , see method) would disperse a larger amount of seeds than animals with lower spatial memory (fast memory decay,  $\beta \rightarrow \infty$ ), (2) in particular in landscape where plants are located at farther distance from each other, because only animals with memory would be able to remember and re-locate both plants. Moreover, (3) animals with higher memory would disperse seeds from both plants evenly, because they would remember the location of both trees and forage from them equally, in particular when these are located farther apart. For the same reason, (4) animals with higher memory would disperse the seeds closer to the parental trees, because they would go to forage more often than an animal with lower memory, increasing the probability that the seed ingested would be deposited closer to the parental tree. If plants are located a larger distance, then the dispersal distance should increase because animals would travel more between the two food sources. (5) Animals with high memory would travel less around the landscape than animals with no memory, because their movement would be less random and more directed towards the food sources and the proportion of landscape travelled would be bigger when plants are further away because the animals would have to travel more distance to forage. At last, (6) animals with high memory would live longer than animals with low memory, because they are better in locating food.

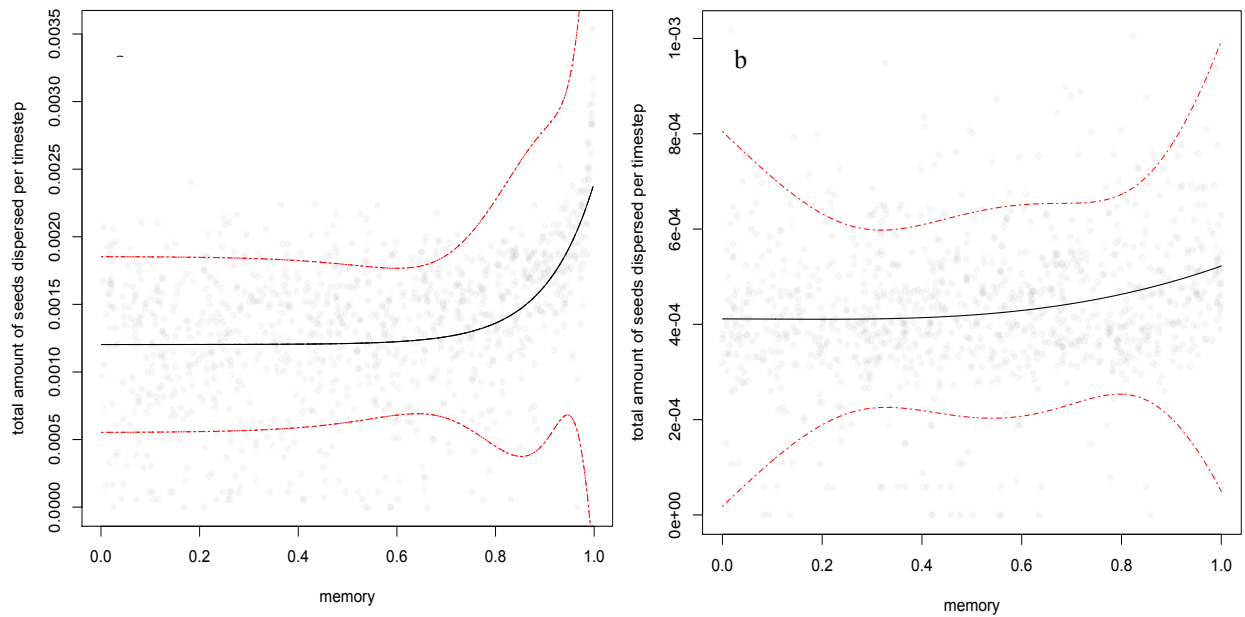
## 2.5.6 Results

### 2.5.6.1 $H_1$ : Total amount of seeds dispersed in the environment

First, the impact of memory on the total amount of seeds dispersed in the environment was analysed. In a landscape where the availability of food was unlimited, results showed that the number of seeds dispersed followed an exponential curve characterised by a flat line at the lower values of memory followed by an exponential increase starting at a value of memory 0.8 (fig 2.4a). This implies that only high levels of memory make a true difference in the amount of seeds dispersed in the environment, while lower and intermediate degrees of memory offer approximately the same results. Animals with the highest memory visited the plants more often (focal plant: mean number of visits = 137.67, sd = 77.55; non-focal plant: mean number of visits = 129.42, sd = 68.39) than animals with low memory (focal plant: mean number of visits = 67.38, sd = 65.32; non-focal plant: mean number of visits = 64.48, sd = 68.54) ( $t(1.26)=15.08, p=0.02$ ).

In environments with limited availability of food, the same trend as before was found (fig 2.4b), but this time the exponential increase is not appreciable because the total amount of seeds available in the landscape is limited. In this case as well, animals with higher memory resulted more efficient in finding the plants (focal plant: mean number of visits = 44.6, sd = 22.94; non-focal plant: mean number of visits = 42.28, sd = 23.71), than animals with lower memory (focal plant: mean number of visits = 27.05, sd = 23.06; non-focal plant: mean number of visits = 27.87, sd = 24.06) ( $t(1.36)=14.27, p=0.02$ ).

The total amount of seeds dispersed in the environment was not affected by the interaction between memory and distance between the plants in either type of landscape.



**Figure 2.4** a) Total amount of seeds dispersed per timestep in environment with unlimited resources by animal with different memory (0= low memory; 1= high memory). b) Total amount of seeds dispersed per timestep in environment with limited resources by animal with different memory (0= low memory; 1= high memory). The red lines indicate the standard deviation.

#### 2.5.6.2 $H_2$ : individual amount of seeds dispersed from the two plants

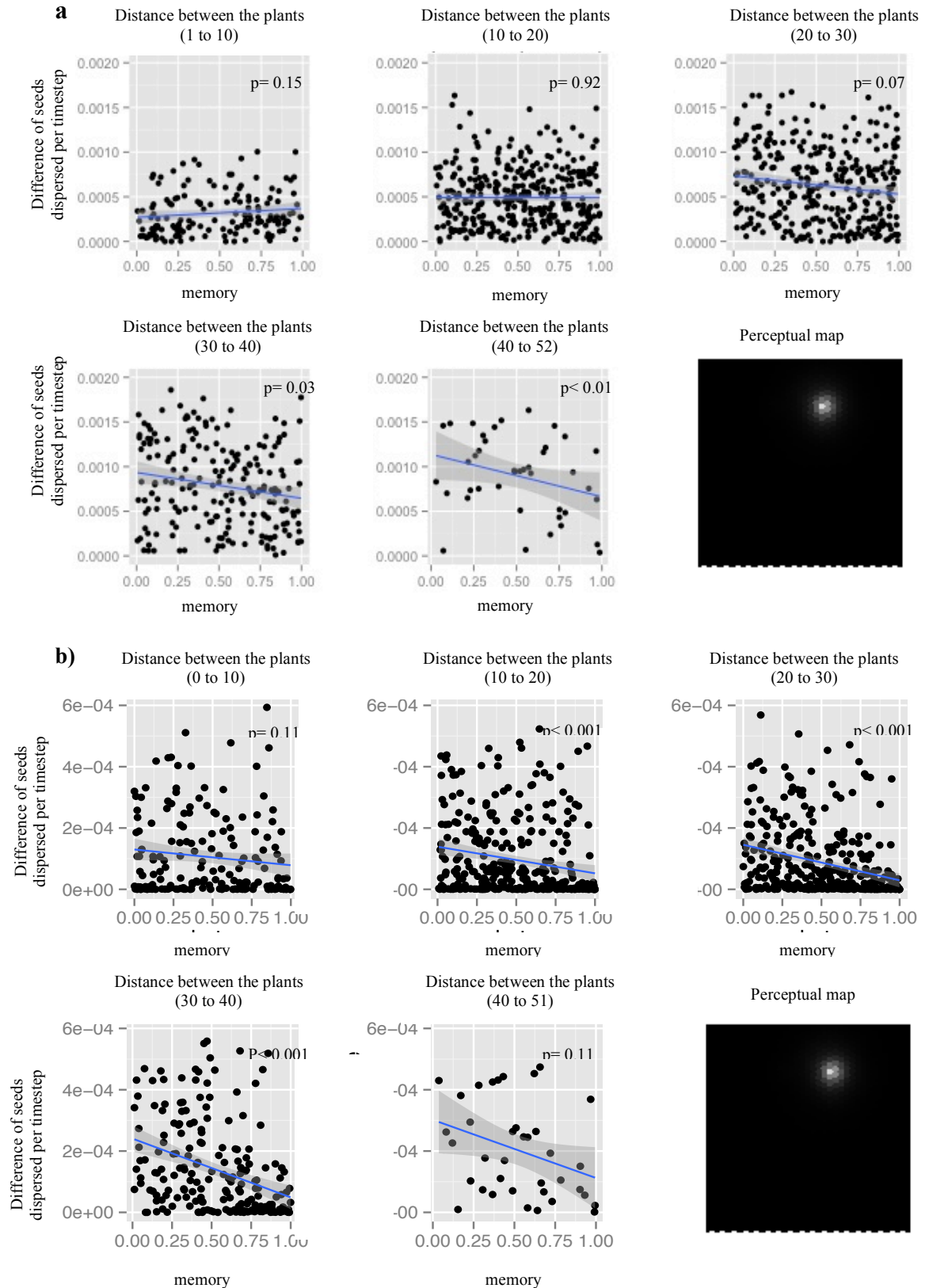
The impact of animals with different memories on the quantity of seeds dispersed from the two trees was analysed, in order to examine whether memory would favour seed dispersal of the known plant or provide equal dispersal from both trees.

The amount of seeds dispersed from the two plants is affected by the interaction between the memory of the animal and the distance between the plants

( $F(1, 997) = 11.86, p < 0.001$ ) in landscape with unlimited availability of food (fig 2.5a). Memory determines the difference in the amount of seed dispersed from the two plants, in particular when the trees are located at a larger distance from each other.

As expected, if animals were able to remember the location of both trees, they fed from them at similar rate, favouring a more uniform removal of seeds from the plants when these were located further apart. However, when the trees were close together, the level of memory had no effect on the relative amount of seeds dispersed from the two plants. This might be due to the fact animals have perception (explained in the method, section 2.5.4) allows them to use information from the surrounding environment within a certain distance. Thus, if the distance between the plants is smaller than animal's perception, the effect of memory is null.

The same results were found in landscapes where the availability of food was limited. An interaction was found between the distance between the plants and animal memory ( $F(1, 997) = 7.12, p < 0.01$ ) (fig 2.5b). In this case the difference of seeds dispersed from the two plants is even smaller, reaching almost zero, because the food sources could be depleted, and animals with high memory tended to consume all the food available in the landscape.



**Figure 2.5** a) Difference of seeds dispersed from the plants in landscape with unlimited food sources at different memory (0= low memory; 1=high memory) and distance between the plants. b) Difference of seeds dispersed from the plants in the landscape with deplorable food sources at different memory (0= low memory; 1=high memory) and distance between the plants. Each point indicates one model iteration (N=1000). The line indicates the average amount of seeds dispersed per timestep  $\pm$  SE. The perceptual map illustrates the amount of landscape (light hexagons) perceived by the animal.

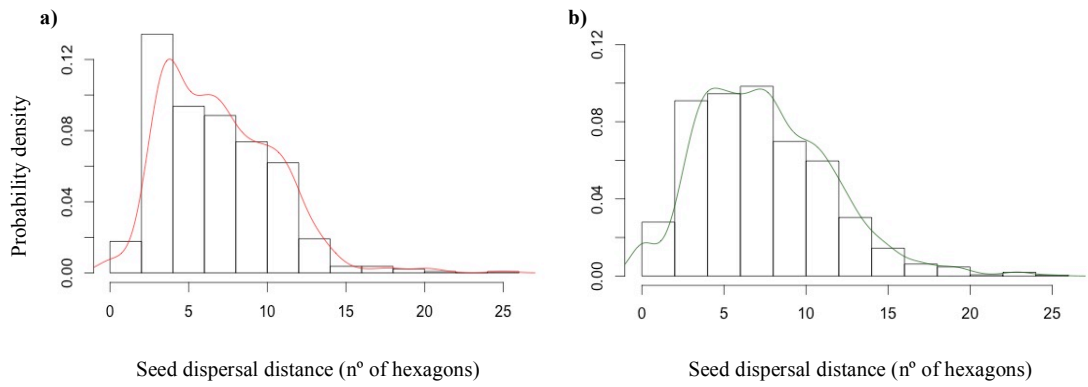


### 2.5.6.3 *H<sub>3</sub>: Long dispersal distance*

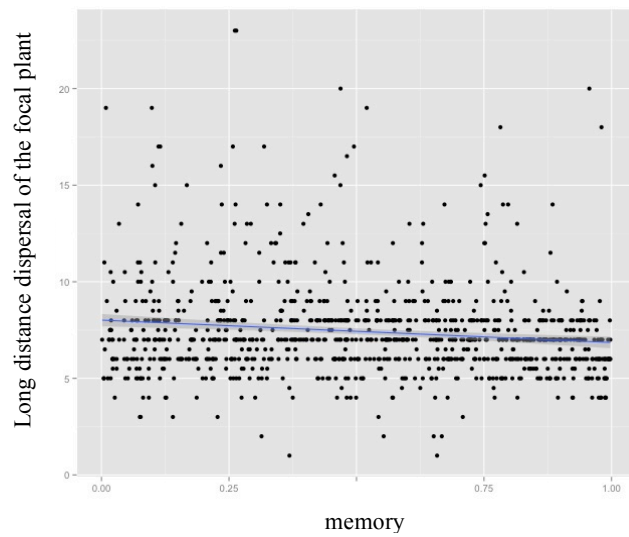
Another important aspect of seed dispersal is the distance to which the seeds are dispersed from the parental tree. According to the Janzen-Connell hypothesis (1970), the further the seeds are deposited from the parental plant the higher is their chance of survival. Therefore, I looked at the seed dispersal distances from animals with different memory.

#### 2.5.6.3.1 In landscape with unlimited availability of resources

**Focal plant:** as regard the focal plant, no interaction was found between animals' memory and distance between the plants ( $F(1, 973) = 0.032$ ,  $p = 0.864$ ). The simulation showed that animals with low memory dispersed seeds at farther distance from the parental tree than animals with high memory ( $F(1, 973) = 17.97$ ,  $p < 0.001$ ). Figure 2.6a, shows that the majority of seeds dispersed by animal with high memory is deposited at less than five hexagons of distance from the parental and decrease almost to zero at a distance of fifteen hexagons. Animals with low memory (fig 2.6b) instead, disperse a higher amount of seeds at a distance of seven hexagons from the parental plant and provide dispersal events at fifteen and above. This might be due to the fact that, as showed previously, animals with higher memory tend to visit the plants more often than animals without memory, thus increasing the chances that the seed would be deposited closer to the parental tree. However, the significance level might be due to the large sample size analysed. In fact, looking at figure 2.7, the distribution of the data looks quite uniform and the linear correlation coefficient approaches the zero ( $r = 0.017$ ).

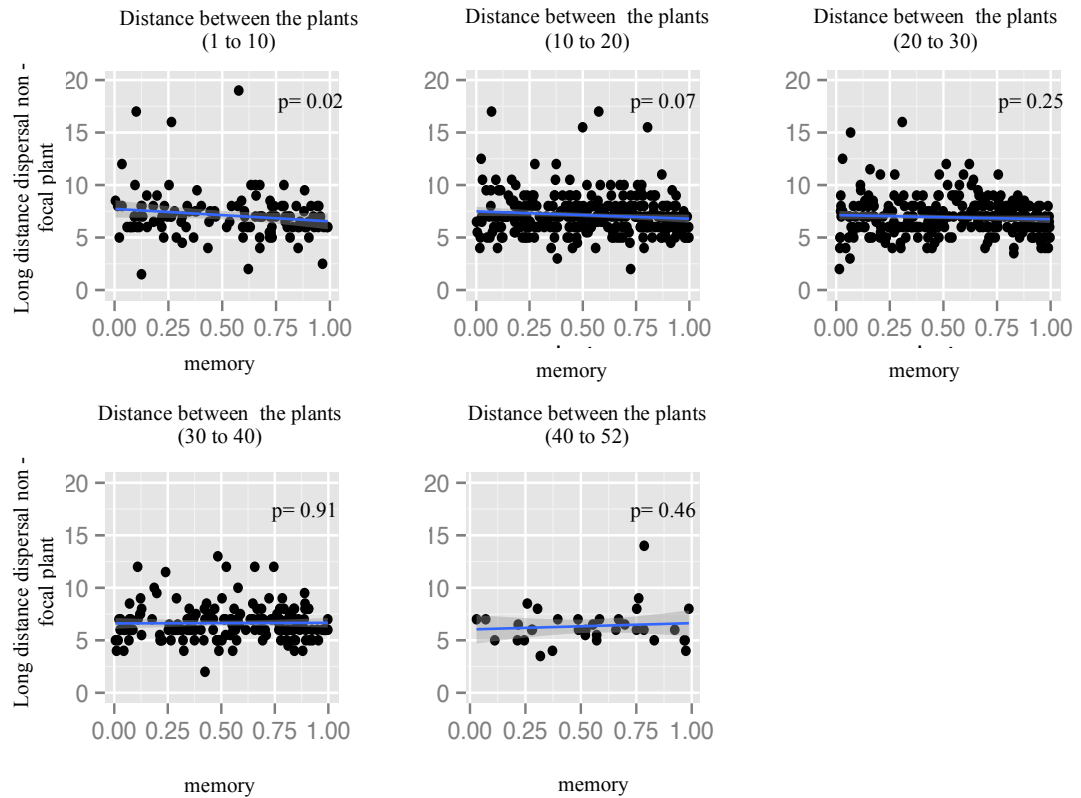


**Figure 2.6** Seed dispersal distance of the focal plant provided by animals with high memory (a) and low memory (b) in environment with unlimited food availability.



**Figure 2.7** Seed dispersal distance (median) at different memory (0= low memory; 1=high memory) of the focal plant in environment with unlimited food availability. Each point indicates one model iteration (N=1000). The line indicates the average amount of seeds dispersed per timestep  $\pm$  SE.

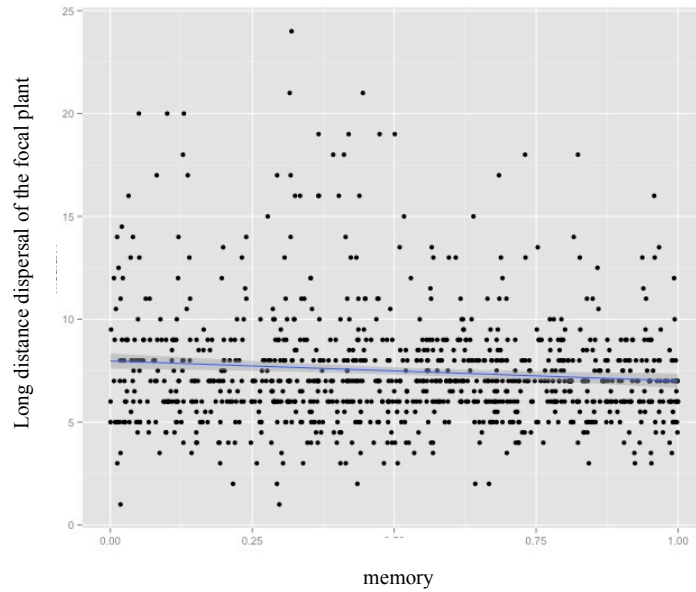
**Non-focal plant:** an interaction between memory and distance between plants was found ( $F(1, 974) = 7, p < 0.01$ ), in which the seed dispersal distance was lower at higher memory when plants were close together (Fig 2.8).



**Figure 2.8** Seed dispersal distance of the non-focal plant in landscape with unlimited availability of resources at different memory (0= low memory; 1=high memory) and distance between plants. Each point indicates one model iteration (N=1000). The line indicates the average amount of seeds dispersed per timestep  $\pm$  SE.

#### 2.5.6.3.2. In landscape with limited availability of resources

**Focal plant:** no interaction was found between animals' memory and distance between the plants ( $F(1,973) = 1.63$ ,  $p = 0.201$ ). In this case the data shows that animal with more memory disperse seeds closer to the parental plant ( $F(1,973) = 9.11$ ,  $p < 0.01$ ), however the linear correlation coefficient approaches zero ( $r = 0.008$ ) (fig 2.9).



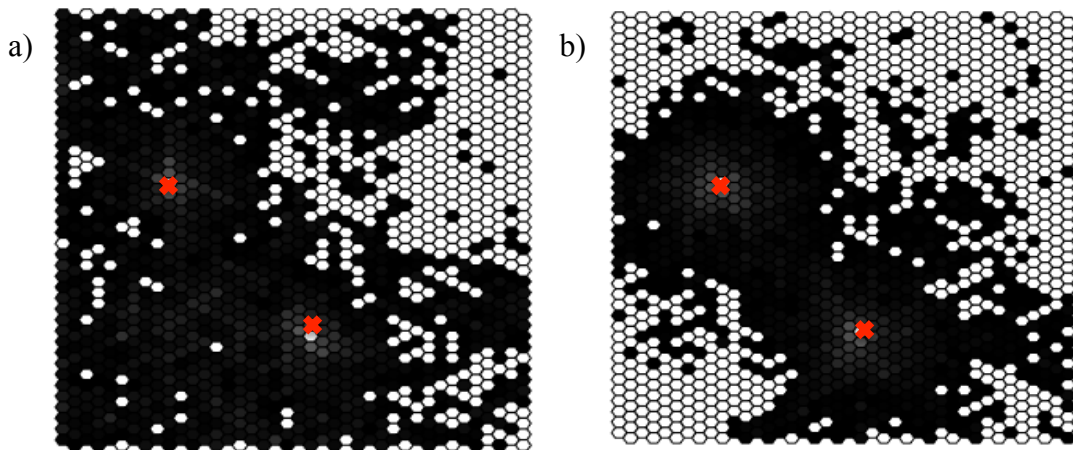
**Figure 2.9** Seed dispersal distance at different memory (0= low memory; 1=high memory) of the focal plant in landscape with limited availability of food. Each point indicates one model iteration (N=1000). The line indicates the average amount of seeds dispersed per timestep  $\pm$  SE.

**Non-focal plant:** no interaction was found between animals' memory and distance between the plants ( $F(1, 974) = 0.086, p = 0.769$ ). Memory does not seem to affect the seed dispersal distance of the non-focal plant ( $F(1, 974) = 3.61, p = 0.057$ ).

#### 2.5.6.4 $H_4$ - $H_5$ : Proportion of landscape travelled

The proportion of landscape travelled was not affected by the interaction between memory and distance between the plants ( $F(1, 997) = 0.93, p = 0.33$ ) and the proportion of landscape travelled by the animals was not related to the memory ( $F(1, 998) = 0.003, p = 0.956$ ) in landscape with unlimited food availability. What makes the difference is that animals with higher memory tended to revisit more

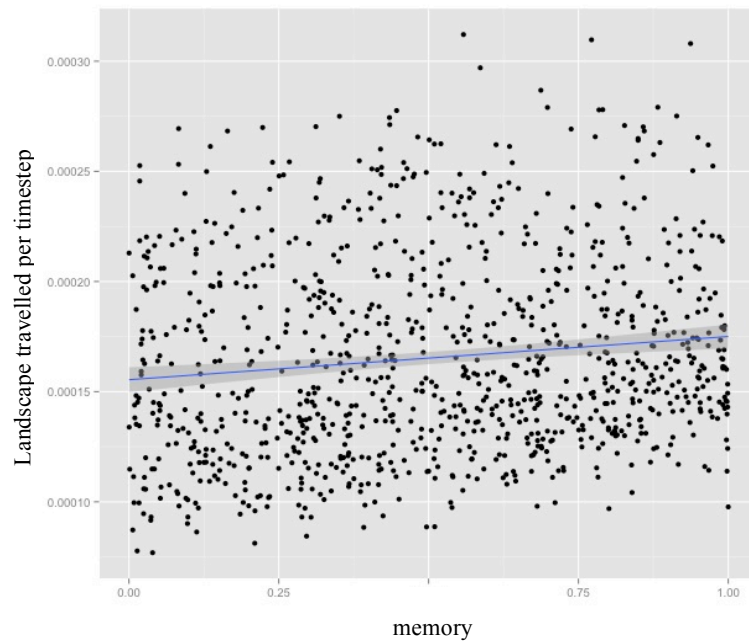
often part of the landscape where the plants are located (Fig 2.10). Thus, even if there is no difference in the amount of landscape explored, memory “constrains” frugivores in certain areas of the environment making their movement less random, and more directed towards the plants.



**Figure 2.10** Examples of landscape travelled by an animal with low memory (a) and with high memory (b) in landscape with unlimited resources availability. In a) and b) the location of the plant is the same. The red ‘x’ indicates the plants. The different shades of grey indicate the number of time that the animal visited the hexagon, the lighter the more the animal visited the hexagon.

In landscapes with limited food availability no interaction was found between memory and distance between the plants ( $F(1, 998) = 2.10, p = 0.147$ ), but the proportion of landscape travelled is higher with higher memory ( $F(1, 998) = 15.97, p < 0.001$ ) (Fig 2.11). This is probably due to the fact that as the resources are depleted, the plant itself becomes less attractive for the animal. Thus, movements of animals with memory are less constrained by the location of the trees, and are therefore prone to explore other parts of the landscape. At the same time, the knowledge of the location of the food source prevents the animal from

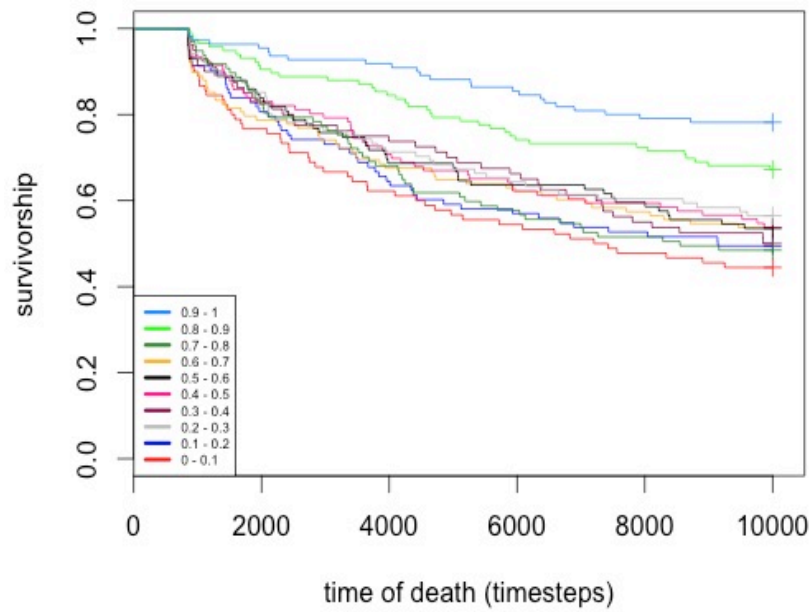
being stuck in a corner of the environment, as happens for animals with no memory.



**Fig 2.11 Proportion of landscape travelled by animals with different memory (0=no memory;1 =high memory) in environment with limited food availability. Each point indicates one model iteration (N=1000). The line indicates the average amount of seeds dispersed per timestep  $\pm$  SE.**

#### 2.5.6.5 $H_6$ : Animal survival rate

Memory was beneficial for animals. In an environment with unlimited availability of food, frugivores with higher memory had better chances of survival than animals with lower memory ( $\chi^2$  (2, N=1000)= 62.72,  $p < 0.001$ ) (Fig 2.12) and no interaction was found between memory and distance between plants ( $F$  (1, 996)= 0.19,  $p = 0.658$ ).



**Figure 2.12 Survivorship rate at different memory (0=low memory; 1= high memory) in landscape with unlimited resources availability. The legend on the bottom- left indicates the range of the values of memory in which the data were grouped exclusively for a clearer representation (0-0.1= low memory; 0.9-1= high memory).**

In an environment with limited food availability, the mortality of the animal is higher in general due to the depletion of the food, which is close to complete depletion when animals possess high memory. Death rate of animals was not affected by the interaction between memory and distance between the plants ( $F(1, 996) = 4.48, p = 0.061$ ), but memory was significant as main effect ( $\chi^2(2, N=1000) = 10.09, p < 0.05$ ). Figure 2.13 shows that this time memory is a disadvantage for the animal, as animals that possess higher memory die faster than animals with low memory. This may be due to the fact that they are better at finding food, and therefore they rapidly depleted the resources.

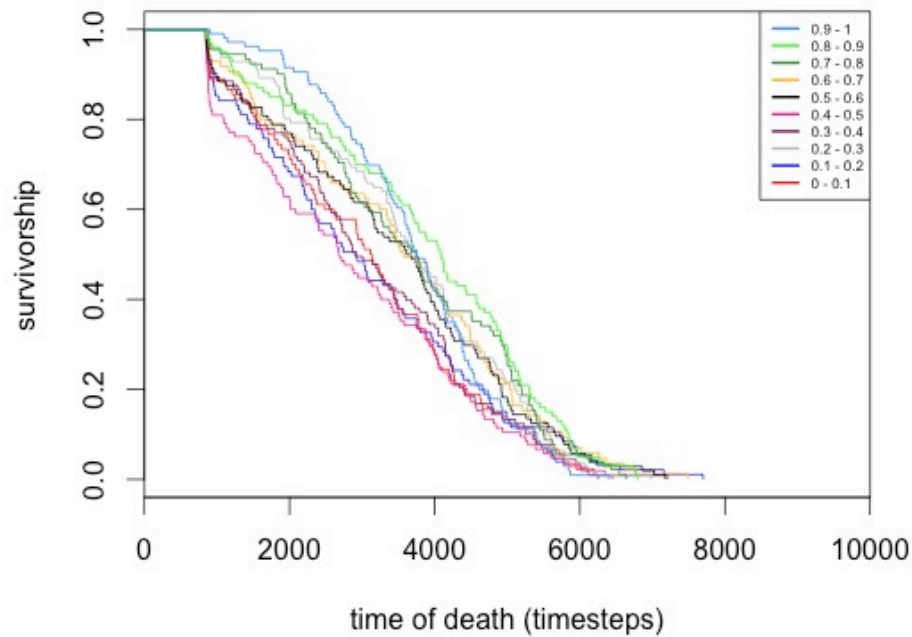


Figure 2.13 Survivorship rate at different memory (0=low memory; 1= high memory) in landscape with limited resources availability. The legend on the top-right indicates the range of the values of memory in which the data were grouped exclusively for a clearer representation (0-0.1= low memory; 0.9-1= high memory).

### 2.5.7 Discussion and impacts on seed dispersal

The model showed how spatial memory could affect the seed shadow in terms of seed dispersal quantity and quality. As regards quantity, as expected animals with better spatial memory were able to relocate the plant more efficiently than animals with lower memory. As a consequence, the amount of seeds removed from the plant and dispersed in the environment, was higher for animals with higher memory. It is worth noting that this increase in the amount of seeds dispersed was not linear, but exponential, so that animals able to retain the information for a shorter time provide the same results as animals that have no memory, and suggesting that exclusively long-term spatial memory could make a big difference in the removal rate of fruits from plants.



Spatial memory was also beneficial in providing a more even distribution of fruit removal from different trees. This depended both on memory, and on the spatial configuration of the plants in the landscape: when plants were close together, memory did not play a role in the inequality of seeds dispersed from trees, which received approximately equal visits. Differences become apparent when the plants were located further away from each other. In this case, animals that were able to remember the location of both trees in the environment tended to visit both plants equally, dispersing a similar amount of seeds from either plants, while animals with low memory visited preferentially one plant over the other causing inequality of fruit removal. This could be due to the fact that when the plants are distant from each other, they cannot both be perceived at the same time, because they are at a distance bigger than the perception field of the animal. As a consequence, only animals that remember the location of both plants forage successfully from either of them. Another explanation is that, once the animal is satiated, it tends to move away from the plant (see method). Once this happens the animal tends to explore the environment until it is hungry again. At this point for animals that are able to remember the location of both trees the choice of one of them would be equal to the 50%, while for animals that have no spatial memory, the choice of the tree would depend on the proximity of the trees to their trajectory, and it is more likely that the closest plant would be the one on which the animals were previously foraging. These results suggest that, if animals' memory skills would not be considered, the spatial conformation of plants would be essential, as the equality of seed removal would depend on the distance between the plants. This is in line with what was found by Carlos and Morales (2008) who investigated the effect of plant aggregation on fruit removal using a

model simulation. Their study showed that as fruiting plants become aggregated, inequalities in fruit-removal rates increase and seed dispersal distance decreases. Both of these processes could help create and maintain plant aggregation and affect genetic structuring. This is the case, for example, for *Miconia fosteri* and *Miconia serrulata* trees and their main dispersers (*Pipridae* sp.) in the tropical wet forest of Ecuador (Blendinger *et al.*, 2008). The model described in this chapter brings these findings a step forward, as it shows that animals' spatial memory skills could reduce the effect of distance between the plants, removing the inequality of seed removal between aggregates of plants and isolated trees: animals with high memory were in fact feeding from both trees in the landscape equally, no matter how far apart they were located.

As regards seed dispersal quality, the dispersal distances at which the seeds were deposited were examined. Not surprisingly, results showed that animals with better spatial memory skills deposited the seeds closer to the parental plant. This is a direct consequence of the fact that they were able to relocate the plant more efficiently than animals with poor memory, increasing the probability that the seeds were deposited closer to their parental tree. In this case memory could be disadvantageous for the plants (Janzen, 1970, Cain *et al.*, 2000, Schupp *et al.*, 2010). However, it is possible that this aspect would be rectified in nature thanks to secondary dispersal events.

Memory was an advantage for animals since it allowed them to forage more efficiently directing their movement towards the food sources and decreasing their randomness of movement. This implies that animals with better spatial memory skills tend to explore the environment differently than animals that move at

random, by visiting preferentially the area of the environment in the proximity of the plants. As consequence, this behaviour would lead to aggregation in the deposition patterns of seeds. This finding is supported with an empirical study on spatial memory in primates (Di Fiore and Suarez, 2007) in which it was found that the repetition of routes between trees results in spots with high seed density. Although this phenomenon makes, to a certain extent, the shape of the seed shadow predictable, it is not advantageous for plants: aggregations of seeds, particularly if they are of the same species, increases competition among seedlings and results in higher mortality (Shupp *et al.*, 2010). Thus, as regard dispersal distances and deposition patterns, animals that move at random in the environment would provide a better service to plants, allowing them higher chances to colonize new areas. Our results are in line to what found by Boyer and Walsh (2010) who suggest that stochastic decisions of animals are still playing a crucial role for the organisms and the environment, as the model they proposed showed that excessive memory could prevent animals from exploring the environment and produce very predictable movements with repeated visits to the same sites (Boyer and Walsh, 2010). Thus, random searches are essential to promote variability in seed dispersal.

Unsurprisingly, better ability in relocating food sources allows animals to live longer. Approximately the 90% of the population with high memory survived for all the duration of the simulation, while for animals with low memory the number decreased at the 50%. However, in the environment where the food sources are depletable, the model shows that animals with higher memory die faster, as their ability to successfully relocate fruiting plants plays against them, making them consume all the available food in a shorter time. However, this situation is

unlikely to be found in nature, where the animals can move to other areas that provide food.

In conclusion, our model showed that trading off between random and memory-based decisions can bring advantages and disadvantages to plants and animals, but most important, that different cognitive skills produce different effects on seed dispersal. What it is sure is that knowledge about the cognition of frugivores could be an effective way to predict more precisely animal choices and movements and the consequences of seed dispersal for ecosystem and communities.

## **2.6 General discussion**

The results of the spatial memory experiment have important implications for seed dispersal: first of all the findings suggest that tortoises tend to visit food sources previously encountered instead of looking always for new ones, enhancing their foraging efficiency. For example, Di Fiore and Suarez (2007), showed this to be the case in spider (*Atheles belzebuth*) and woolly monkeys (*Lagothrix poeppigii*), which follow repeatedly feeding routes. Tortoises were to remember the location of food for a period of two months, which is equivalent to the duration of the production of fruit of some tropical plants (Moskovitz and Bjorndal, 1990). Moreover, they proved to be able to adopt a large variety of navigation strategies thus they are likely to be able to orientate in a variety of settings. This plasticity may be advantageous for tortoises, because it would allow them to relocate food sources even in case of changes in the environment, and

also for the plant, because it would increase the probability that the fruit would be removed by a disperser instead of seed predators. Moreover, it would increase the chances that the tortoises would choose a different path to reach the food locations, which would avoid the creation of aggregates of deposited seeds, as it happens with primates (Di Fiore and Suarez, 2007). However, studies on the movement of the red-footed tortoise in the field did not report trajectories undertaken by these animals, but only daily activities patterns (Moskovitz, 1987; Jerozolinski, 2009; Montaña *et al.*, 2013). Thus, to date, there is not enough information available to confirm whether *C.carbonaria* actually uses its potential to relocate food sources in its own environment. To my knowledge, evidence of the use of spatial memory skills to relocate fruiting trees in the wild comes exclusively from studies on primates (e.g. Janmaat *et al.*, 2010; Di Fiore and Suarez, 2007; Janmaat *et al.*, 2013).

More information about frugivores spatial memory skills are essential because, as highlighted by the seed dispersal model, there is the evidence that animals with different spatial memory skills bring different kind of services to the seed dispersal processes. Thus, knowing for how long an animal is able to remember the location of a food source, would give us the possibility to predict which type of impact it would have on seed dispersal in its natural environment. The model was designed to test exclusively the effect of long-term memory on the seed shadow of trees, thus it deliberately represented a simple environment with no other factors than memory affecting the movement of the animals. I am aware than in natural environment the location of food is not the only variable to consider in order to predict where an animal would go, but it wanted to explore how considering animal cognition in mechanistic models would improve the

accuracy of the results. As suggested by Cousens *et al.*, 2010, the possible key to a deeper understanding of seed dispersal by endozoochory would be to address the attention on what determines the behaviour of the animals, instead of limiting the simulation to a behaviour of the vector in a specific case of study. Thus, it can be concluded that better knowledge about the spatial memory of frugivores will definitely help to create more reliable predictions about the seed shadow of trees.

# CHAPTER 3

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## 3. WHAT?

### 3.1 Experiment 3: motivation and food choice in *Chelonoidis carbonaria*

#### 3.1.1 Introduction

As explained in the general introduction, perceived quality and quantity of food are two of the benefits that are likely to motivate frugivores to choose some fruit instead of others. From the point of view of the plant, animals' motivation to feed on its fruits would increase the amount of seeds removed and dispersed, which is translated into an increase in the plant's fitness (see chapter 1 section 1.4.2).

Summarising what was mentioned in section 1.4.2 about animals' perception of food quality, in a controlled environment where the costs associated with the food sources are negligible, this could depend on its taste (Yarmolinsky *et al.*, 2009; Zhao *et al.*, 2010) and digestive feedback (Yearsley *et al.*, 2006; Werner *et al.*, 2008), which may or may not be related to the nutritional content of the fruit. Reptiles, as with many other species investigated, have food preferences that might be based on the content of phosphorus, minerals and the ratio between

calcium and phosphorus (Moskovitz and Bjorndal, 1990). This means that in a scenario in which they are allowed to choose between different types of food, they eat first the preferred ones (e.g. Squamata: Christian *et al.*, 1984; Duarte da Rocha, 1989; Godínez-Álvarez, 2004; Chelonia; Grassman and Owen, 1982; Moskovitz and Bjorndal 1990; Rall and Fairall, 1993; Crocodilya: Borteiro *et al.*, 2009; In particular food preferences of red-footed tortoises are described in Moskovitz and Bjorndal, 1990). Thus, from point of view of a plant, being preferred by frugivores is an advantage because it means having more chances to have the fruits quickly removed. For example, Vilà and D'Antonio (1998) showed that, in northern California, plants with the preferred fruits (*Carpobrotus edulis*) receive a faster fruit removal than the less preferred *Carpobrotus chilensis*. Nonetheless, the total fruit removal at the end of the season was approximately the same for both species. However, studies show that animals' fruit preference might change according to the cost associated with accessing the food source, such as distance from the individual (Levey *et al.*, 1984), or the presence of predators (Kacelnik *et al.*, 2013) etc (see 1.4.2). This implies that it is necessary to evaluate both the costs and benefits associated to a particular food source, in order to have a better indication of what impact there might be on the foraging decision of the animals and the strength of the motivation of the animals to choose and feed on particular fruits.

Food quantity, as mentioned in the general introduction (section 1.4.3), is another important benefit, other than quality, of a food source, as it contributes to the growth and fitness of animals (Pyke, 1984; Guisande, 2000; Cruz-Rivera and Hay, 2000). Therefore animals able to determine food patches with the largest amount of food would be advantaged (Dehaene, 1997). Quantity discrimination, is



an ability possessed by a variety of taxa (mammals: Addessi *et al.*, 2008, Ward and Smuts, 2007; birds: Al Aïn *et al.*, 2009; amphibians: Krusche *et al.*, 2010), however, it has not been yet investigated in reptiles. An interesting outcome of quantity discrimination studies is that animals seem to be biased towards items larger in volume, even when the alternative choice has a bigger total amount of food but split in smaller parts (e.g. Boysen *et al.*, 2001; Beran, 2008). However, this effect disappears when items are replaced by symbols (Silberburg and Fujita, 1996). Thus, it looks like animals have an innate response to larger volumes of food (Kaufman *et al.*, 1996; Beran, 2008). It could be hypothesised that preferentially choosing foods that are larger in volume might be adaptive, as it maximizes the amount of food gained for every foraging bout (Pyke, 1984). This leads to the question as to whether frugivores would be more motivated to forage from plants that offer larger amounts of smaller fruits or fewer fruits that are larger in size. Sallabanks (1993) observed that American robins (*Turdus migratorius*) tend to have a hierarchical choice of food when foraging, selecting, at first, shrubs with the biggest amount of fruits and then choosing among fruits according to their size by favouring the largest ones. Another study on the fruit size of the plant *Ocotea tenera* showed that between plants of the same species the ones with greater than average sized fruits received more visits from birds, and as a consequence a higher fruit removal rate, than plants with smaller fruits (Wheelright, 1993). Thus, if this tendency of selecting large size fruit between the plants of the same species is spread among frugivores, it might be hypothesized that plants that produce larger fruits would attract more seed dispersers and have higher fruit removal rate than plants with smaller fruits.

The aim of the present study was, therefore, to test whether a species of

reptile, the red-footed tortoise, was able to discriminate between different volumes of food. This would (1) contribute to the literature on animal quantity discrimination, adding information about reptiles, and (2) investigate whether frugivores, other than primates and birds, tend to choose fruits of bigger size. In order to avoid the possibility that the choice of tortoises was biased by the view of larger volumes of food, as in the case of chimpanzee (Beran, 2008), tortoises were not allowed to directly see (or smell) the food, but the different rewards were replaced by coloured cues (see the methods 3.1.2). Moreover, to evaluate the relative potency of tortoises' choice, the cues were presented at different distances from the tortoises. Distance represents a cost associated to the food, as it is proportional to calories consumed, and could alter the choice of food of individuals: birds, for example, chose to feed from the less preferred fruit when closer to them, even if the preferred one was available but further away (Levey *et al.*, 1984). As a further control, the same procedure was repeated using two different perceived qualities of food.

### **3.1.2 Method**

#### *3.1.2.1 Phase One*

Subjects were four captive juvenile red-footed tortoises with carapace length between 7.7 and 9.3 cm. When the tortoises were not occupied in the experiment, they were housed all together in an enclosure of 1m x 1.5m with water ad libitum, shelters and a hot spot with heat and a UV lamp. The lights were kept on a 12 hour cycle; the substrate was covered with bark that was damped once a day. The temperature of the room was kept between 27 and 30 degrees °C.

Tortoises were fed once a day after the completion of their daily training, with a variety of fresh fruits, excluding mango and apples, that were used as rewards during the experiment.

Tortoises were trained and tested in an experimental arena measuring 1m x 1m, with the floor entirely covered by sand (5cm deep) in order to increase their difficulty to walk. The arena was located in the same room where the tortoises were housed.

#### *3.1.2.2 Habituation phase*

Each subject was habituated to the arena prior the start of the experiment. Tortoises were individually introduced in the experimental arena for a period of 20 minutes two times on two consecutive days. During this time, they were free to explore the environment with no restrictions. On the third day, eight pieces of food were scattered throughout the arena. If the tortoise ate at least five pieces of food it was considered habituated and ready to start the training phase. In the opposite case, the tortoises received further habituation sessions as necessary.

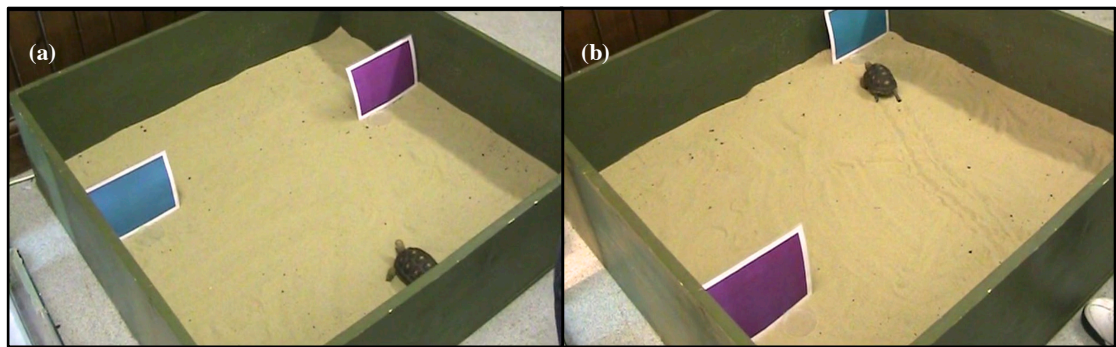
#### *3.1.2.3 Phase One Training*

The reward used in this phase was mango jelly. This was made by mixing mango juice with powdered agar in a 100:1 ratio. The same batch was used throughout to control for biases due to texture or ripeness of fruit. In this way, the quality of the reward was kept constant throughout the experiment.

The tortoises were trained to associate two different coloured stimuli to two different quantities of food reward (counterbalanced across individuals). The stimuli were laminated sheets (10.5 x 14.85 cm) of two different colours, turquoise and purple. Previous research has shown that tortoises are able to discriminate between these colours (Burghardt, 1977). The rewards differed in quantity (cubes with side length of 5mm<sup>3</sup> and 3mm<sup>3</sup>) but not in quality (all mango). During the training phase a single stimulus was presented to the tortoise at a distance of 30, 60 or 90 cm from the starting position and to the left, right or middle relative to the starting point. This provided the tortoises with experience of distance, position and reward type prior to testing. At the start of each trial the tortoise was placed in the arena inside a small cage to allow it to observe the stimulus. Once the tortoise had oriented towards the stimulus for at least five seconds it was released. A choice was considered as an approach to within 3cm the stimulus, with the head turned towards it. After a choice was made a piece of mango jelly was dropped in a transparent bowl positioned in front of stimulus and the trial ended. Tortoises were removed from the arena as soon as they had eaten the jelly. The subjects had one minute to complete the trial starting from their first movement. If they did not complete the trial within this time period they were removed from the arena and the trial was repeated after two minutes. If they did not move, they were removed from the arena after five minutes, and the trial was repeated after two minutes. The arena was cleaned and the sand substrate mixed between each trial to remove possible scent traces left between trials. Tortoises received one training session per day, which consisted of six trials. After the completion of 72 trials (four for each stimulus and position) the subjects proceeded to the test phase.

#### 3.1.2.4 Phase One Test

During testing both stimuli (turquoise/purple) were presented at the same time; each stimulus could be located at 30, 60 or 90 cm, either to the left or to the right of the starting point (Fig. 3.1a, b). Therefore 18 stimuli combinations were possible. As during training, tortoises were introduced into the arena inside a small cage to ensure that they had time to visualize both stimuli prior to release. When the tortoise approached one of the two stimuli, it was rewarded with the corresponding reward, and removed from the arena as soon as it had consumed the jelly. In all other ways the tests were the same as the training trials. Each tortoise completed 90 test trials (five trials for each combination of distances).



**Figure 3.1.** Experimental setup of Phase One: examples of possible combination of distances of the stimuli. In (a) the stimuli are located at the same distance (60 cm) from the starting point. In (b) the stimuli are located at different distances (30cm, purple; 90cm, turquoise) from the starting point. In this example the tortoise had the combination turquoise= cube with side length of 5mm<sup>3</sup> of mango jelly and purple= cube with side length of 3mm<sup>3</sup> of mango jelly.

#### 3.1.2.5 Phase two

Six captive juvenile red-footed tortoises (four of them were the same tortoises as were used in the phase one) with carapace length between 7.7 and 9.3 cm, were used in this phase of the experiment.

#### *3.1.2.6 Phase Two Training*

Two different rewards were used in this phase: mango jelly and apple jelly, made following the same procedure described above. Before starting the experiment, preference tests were given, in which the jellies were presented to each tortoise on two petri dishes at a distance of 20cm one left and one right at random in three different occasions. All tortoises ate the mango jelly first, and approached the apple jelly once the mango was finished. Mango jelly was therefore considered to be the preferred reward. The arena and the procedure of the training and test phase were exactly the same as in the phase one. However, the tortoises were trained to associate two novel stimuli of different colours (blue and orange) to two different reward types (counterbalanced across individuals). The rewards, therefore, differed in perceived quality (mango or apple jelly) but not in quantity, as the rewards were always cubes with side length of 5mm<sup>3</sup>.

#### *3.1.2.7 Phase Two Test*

The test performed was exactly the same as in phase one, but using the colour stimuli indicating mango and apple jelly.

The experimenter was in the test room during the whole duration of training and testing of phase one and two, located exactly behind starting point of the tortoises. Training and test trials were recorded with a camcorder (Canon Legria) located on the left of the experimenter (fig. 3.1)

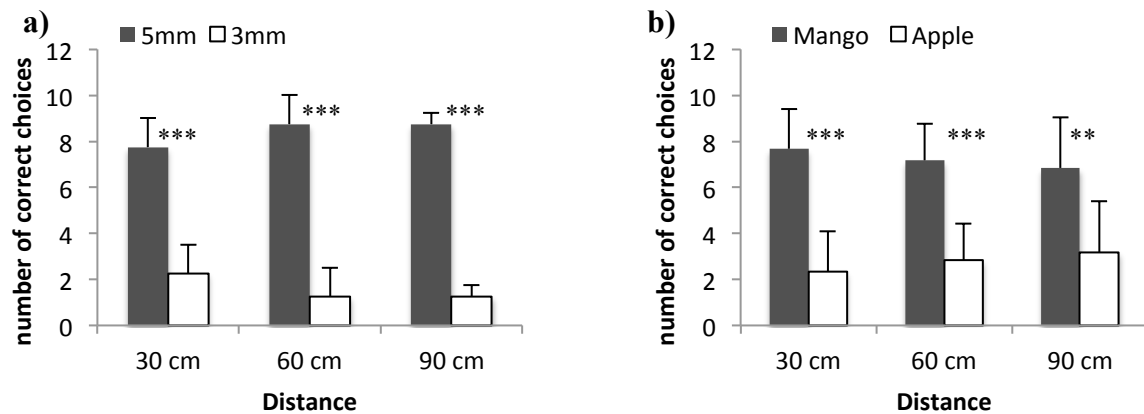
### *3.1.2.8 Statistical analysis*

To investigate the choice of the tortoises and whether distance impacted upon choice of food of the tortoises a generalized linear model with a poisson error distribution was used, using the glmer function in R 2.15.0. For Phase One the volume of jelly (cubes with side length of 5mm<sup>3</sup>/3mm<sup>3</sup>) and the interaction between the volume of jelly and position (distance of the stimulus from the starting point, 30/60/90 cm) were entered as fixed factors and subject identity as a random effect. Significance was assessed by comparing the full model to a reduced model lacking the term of interest using likelihood ratio tests (Crawley, 2005). The same statistical analysis was applied to data collected during the Phase Two. This time, the fixed factors were the type of jelly (apple/mango) and the interaction between type of jelly and the position of the stimulus.

## **3.1.3 Results**

### *3.1.3.1 Phase One*

The tortoises were able to learn the association between colour of the stimuli and volume of jelly, showing a strong preference for the stimulus associated with the larger volume of food when both stimuli were located at the same distance from the starting point (Fig. 3.2a) or the stimulus for the largest volume was closer ( $\chi^2(1) = 15.50$ ,  $p < 0.001$ ) (Fig. 3.3a, b). The tortoises continued to choose the largest reward even when the stimulus was the furthest (90cm) from the starting point ( $\chi^2(1) = 4.00$ ,  $p = 0.04$ ; Fig. 3.3c, d). The position of the stimuli did not influence choice of tortoises (interaction:  $\chi^2(2) = 1.33$ ,  $p = 0.51$ ).

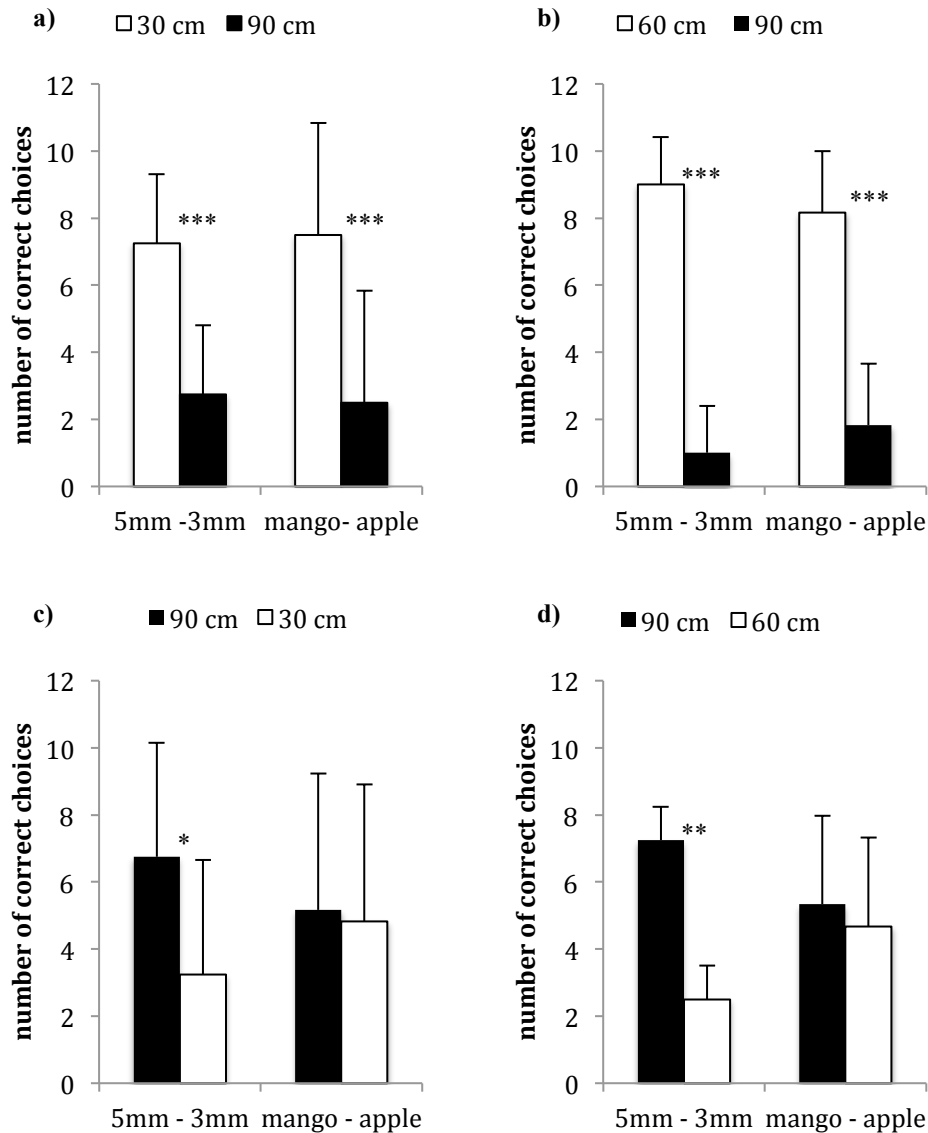


**Figure 3.2** Tortoises' mean  $\pm$  SD correct choices when (a) the stimuli associated to different amount of food (cubes with side length of 5mm<sup>3</sup> and 3mm<sup>3</sup>) or (b) different types of food (mango; apple) were located at the same distance. Asterisks indicate significant differences between groups: \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

### 3.1.3.2 Phase Two

As expected, tortoises showed a strong preference for mango jelly when both stimuli were located at the same distance (Fig. 3.2b) and when mango was closer to the starting point than apple ( $\chi^2(1) = 12.76$ ,  $p < 0.001$ ) (Fig. 3.3a, b). However, unlike Phase One, when the stimulus for mango jelly was farther than for apple (Fig. 3.3c, d), the tortoises' preference for mango dropped to chance ( $\chi^2(1) = 0.14$ ,  $p = 0.71$ ). The position of the stimulus of the preferred reward influenced the choice of the tortoises (interaction:  $\chi^2(2) = 15.00$ ,  $p < 0.001$ ).





**Figure 3.3.** Tortoises' mean  $\pm$  SD correct choices when the stimuli were located at different distances. In (a) and (b) the stimuli associated to the preferred rewards (mango or cube with side length of 5mm<sup>3</sup> of food) were closer; in (c) and (d) the stimuli associated to the preferred rewards were farther away. Asterisks indicate significant differences between the groups: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

### 3.1.4 Discussion

This experiment revealed that a species of reptile can discriminate between different amounts of food: tortoises preferentially approached the stimulus associated with the larger volume of food, regardless of distance. This supports the hypothesis, introduced in section 1.5.3, that the ability to discriminate between

volumes and the preference for larger ones evolved early in the evolutionary scale (Boysen *et al.*, 2001; Beran, 2008), or evolved independently multiple times, suggesting that this skill is adaptive and perhaps used by animals in order to optimise their foraging efficiency.

Tortoises also showed interesting differences in choice behaviour when presented with different quality or quantity of reward. The results showed that their preference for the larger amount of food remained unaltered over distance, whilst their choice of mango changed and dropped to chance when it was located further away than the apple jelly. Similar results were observed in birds, which have been shown to change their food preferences with an increased distance to the preferred fruit (Levey *et al.*, 1984). This suggests that larger amounts of food exert a stronger response compared to a preferred type of food, as tortoises overcame the cost of distance in order to obtain more food but they did not do the same for the preferred food. These findings are in line with what has been found in lizards (Kaufman *et al.*, 1996), which chose the largest snail in spite of the higher handling cost.

However, this behaviour could depend on the quality of the particular reward used: even though mango was strongly preferred to apple when they were located at the same distance, it may be that this preference was not a sufficiently strong incentive for tortoises to incur the cost of walking the extra distance. So, the possibility that different types of reward might influence tortoise behaviour differently cannot be excluded. It seems therefore that the red-footed tortoise is able to consider more than one parameter at time when foraging, and to modulate its decisions according to cost (distance) and the benefits (reward) it can obtain.

Moreover, results imply that tortoises were able to learn the association between a visual cue and the corresponding quantity or quality of food. During the experiment the subjects received the reward only after choosing the stimulus, therefore they learned what the colour indicates and their choice was not biased by the view or smell of food. Thus, they were able to use visual stimuli to infer information about food sources and then choose between them. This implies also that tortoises are able to determine the value of food items from distance, which would improve their foraging strategy (Pyke, 1984).

### **3.1.5 Implications for seed dispersal**

The results of this experiment could have important implications for seed dispersal: first of all, it could be expected that, among plants of the same species, tortoises would direct their foraging towards trees that produce bigger fruits, as they showed good ability to discriminate between quantities and they preferred the largest one. This agrees with what found in Wheelwright (1993): data in the field showed that among plants of the same species (*Ocotea tenera*), the one with larger fruit were visited more frequently by birds and had a faster fruit removal rate than the ones with small fruit. Thus, the volume of fruits seems to be an important parameter in the selection process of fruit by frugivores, impacting on the probability of a fruit of being eaten.

Second, the results support the hypothesis that the location of food sources with respect to other fruiting plants may influence the success of fruit removal (Levey *et al.*, 1984; Fragoso *et al.*, 2003; Hampe *et al.*, 2008). High perceived quality plants and/or plants with bigger size fruits (preferred plants) are likely to

attract more seed dispersers than neighbouring plants with lower quality/quantity fruits (non-preferred plants). This might have two effects: (1) non-preferred plants would suffer lower fruit removal than preferred ones, thus suggesting that fruits of non-preferred plants could be removed faster if isolated than if they were closer to preferred plants (Levey *et al.*, 1984). (2) On the other hand, the competition for the fruits of the preferred plant might represent a cost for some frugivores (Bekoff *et al.*, 1999) which would diverge their foraging to the neighbouring non-preferred plants. Thus, in this case, being next to an attractive plant would increase the chances of fruit removal than being isolated.

## **3.2 Experiment 4: memories of quantity and quality**

### **3.2.1 Introduction**

The experiment described in the previous section (3.1) showed that tortoises are able to discriminate between quantity and perceived quality of food; given the two kinds of jelly, tortoises were more motivated by the volume of food provided than the perceived quality as the subjects were willing to travel further in order to obtain a larger reward rather than a preferred one. Since animals' ability to store information may decay over time (Pyke, 1984), a following question to investigate was for how long can red-footed tortoises remember the associations (colour-type of food) previously learned. This is important because it could be hypothesised that remembering information about the features of a food source might be adaptive: it would in fact help the animal in making decisions on where

to go foraging, without the necessity to re-evaluate the food sources every foraging event and reducing the risk to re-visit food sources that do not offer satisfying food.

Long-term memory in visual discrimination tasks has been investigated in a variety of species; for example pigeons remembered 800 different pictures for a period of 5 years (Fagot and Cook, 2006), sea lions were able to solve a visual task after a 10 year interval (Reichmuth-Kastak and Schusterman, 2002) and elephants were successful in a discrimination task after a lag of 8 years (Stevens, 1978). This cognitive ability in chelonians was recently investigated in terrapins (*Pseudemys nelsoni* and *Trachemys scripta*) which were able to discriminate between a black and white bottle, one of which was associated with food, and remember this visual task for a 3.5 months retention period (Davis and Burghardt, 2012). Long-term memory of *Pseudemys nelsoni*, was proven to be longer than that, considering that they remembered the motor action to solve a specific task after two years retention time (Davis and Burghardt, 2007). Other examples of how some species of Chelonians excel in long-term spatial memory were mentioned in chapter 2 (Rowe *et al.*, 2005; Mitrus, 2006; Wilkinson *et al.*, 2007; 2009; Muller-Paul *et al.*, 2014).

As regards frugivores, whether their motivation to visit certain food sources is maintained thanks to their long-term memory skills, would impact differently on seed dispersal. The experiment 3 showed how being motivated from particular fruit traits (in this case the volume of food) might potentially affect the fruit removal rate from different trees impacting also on the neighbouring plants (Levey *et al.*, 1984; Fragoso *et al.*, 2003; Hampe *et al.*, 2008). Going a step further, these foraging events would impact on plant

dynamics differently if they were constantly repeated, due to the fact that frugivores remember and decide to forage from a specific tree, or if they were the result of a single random event, in the case that animals do not remember information about fruits, thus foraging on the same plant only when occasionally re-encountered in the environment (Boyer and López-Corona, 2009).

Therefore, the long-term memory of red-footed tortoise regarding the association between a visual cue and type of food (quantity and perceived quality) was investigated after a retention time interval of 18 months, thus for a period longer than a fruiting season (Moskovitz and Bjorndal, 1990). It must be underlined that usually, in visual discrimination studies, the animals learn to associate a positive stimulus with the food, while nothing to the negative stimulus, thus the subjects have a strong motivation to learn and remember the correct association, otherwise they would not receive any reward (Fagot and Cook, 2006; Davis and Burghardt, 2007; 2012). In experiment 3, both visual cues provided the tortoises with food, thus it might be expected that their motivation in remembering which cue was providing the better reward might be less strong, because the subject received a reward no matter its choice. Moreover, the few studies in literature that investigated the ability of animals to remember the mental representation of quantity, limited the retention time from few seconds (Ward and Smuts, 2007; Mahamane *et al.*, 2014) to minutes (Beran and Beran, 2004). Thus, to my knowledge, this is the first study that tests long-term memory of mental representation of quantity and perceived quality in reptiles over such a long time interval.

### **3.2.2 Method**

#### *3.2.2.1 Pre-test*

The same tortoises that took part in the experiment described in the previous section (3.1), took part in this experiment. A week before the scheduled date of the test, each tortoise received a pre-test session, during which they had to approach a visual cue in order to gain a reward. This procedure was performed to ensure that the subjects habituated again to the experimental environment and associated the environment with the task of approaching a cue to receive food. The pre-test took place in the same arena, with the surface covered by sand, where the tortoises performed the previous experiments (section 3.1). The procedure was exactly the same as described in phase one of experiment 3 (section 3.1.2). The only difference was the reward, which consisted in a small piece of dandelion, and the visual cue, which was a yellow cylinder. The reward and the visual stimulus used were different to those used during the training and test in experiment 1 (section 3.1.2) in order not to give the tortoises any additional training, thus not affecting the performance of the tortoises during the test. The pre-test ended when the tortoises complete a session of six consecutive correct trials, i.e. the subject approached the cue.

#### *3.2.2.2. Test*

After 18 months from the last day of the previous quantity and quality test respectively (section 3.1.2), tortoises were tested in a two choice task. Both tests for quantity and quality long-term memory discrimination were performed in the

same way. The visual stimuli (turquoise and purple for quantity and orange and blue for the perceived quality) were located in the arena at the same distance, 60cm from the starting point, one left and one right at random. As during the initial training (phase one, section 3.1.2.1), tortoises were introduced into the arena in a small cage and they were released after they looked at both stimuli, to be sure that they were aware of both choices. Then, they had one minute to approach one of the two stimuli. When a choice was made, with the tortoise was at 3cm distance from the cue looking towards it, the subject was removed from the arena without receiving any reward in order to not effectively re-train. After two minutes the next trial started. The test consisted of one session of six consecutive trials. As the stimuli were presented at the same distance, so the cost of distance was null, I expected that if the tortoises remembered the association visual cue- type of food, they would have chosen the visual cue indicating the preferred reward above chance. If tortoises did not remember the association visual cue- type of food, I expected their performance to be at random.

### *3.2.2.3 Data analysis*

The data were analysed using the 1-sample Poisson rate test using Minitab 17. This test is used to estimate whether the rate of occurrence of an event differs from a reference value. Thus, the number of choices of the bigger amount of food for the quantity test, and the choices of mango for the quality test, were introduced as the success events. Since for each trial the probability of being successful was 0.5, and tortoises received a total of six trials, the reference value was set as three. The alternative hypothesis tested was that the rate of occurrence



of the success was greater than the hypothesized rate of occurrence (i.e.,  $> 3$ ).

### 3.2.3 Results

Tortoises successfully remembered the association between the visual cue and the quantity of the reward by choosing above chance the stimulus that represented the large amount of mango (1-sample poisson rate:  $p= 0.037$ ; Fig. 3.4a). Also regarding the perceived quality tortoises showed to possess long-term memory, choosing above chance the cue associated to the mango reward rather than apple (1-sample poisson rate,  $p= 0.028$ ; Fig. 3.4b). In table 4 are reported the individual choices during the quantity and quality test.

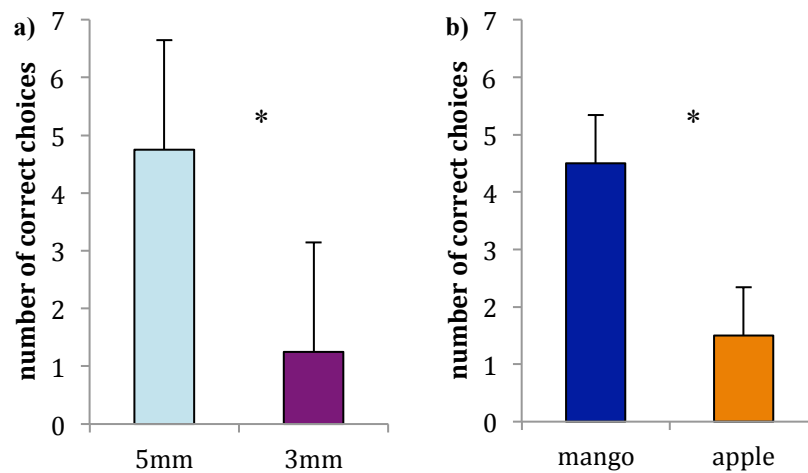


Figure 3.4 Tortoises' mean  $\pm$  SD correct choices after 18 months retention time in the quantity (a) and quality (b) discrimination test. The asterisk indicates the significant differences between the groups,  $*p<0.05$

**Table 4 Individual data of quantity and quality discrimination test.**

Subject	Quantity test		Quality Test	
	5mm <sup>3</sup> Mango	3mm <sup>3</sup> Mango	Mango	Apple
Esme	6	0	5	1
Quinn	5	1	5	1
Timothy	6	0	4	2
Marshall	2	4	3	3
Russell	/	/	5	1
Margot	/	/	5	1

**Table 4. Tortoises' choices during the quantity and quality discrimination tasks; Dashes indicate that the tortoise did not participate to the experiment.**

### **3.2.4 Discussion**

Red-footed tortoises remembered the association between a visual stimulus and the amount and/or perceived quality of food associated to it after a retention time of 18 months. This supports the hypothesis in Davis and Burghardt (2012) that suggested that long-term memory in chelonian should be adaptive, maybe because of their longevity, and they are potentially excellent models for the study of animal memory. The present results add a piece more to the study of animal long-term memory, as it showed that tortoises were able to remember the association between visual cue-feature of the food, instead of exclusively visual cue – food (Fagot and Cook, 2006; Davis and Burghardt, 2012). This means that the type of reward motivates the animal in the selection of food sources in a way that could be comparable to the presence or absence of food and prove that tortoises are able of memories of feeding experience (Ban *et al.*, 2014). A next step would be to investigate how tortoises use other features, such as scent or

texture, to select fruit as common among primates (Dominy, 2004) and to verify which feature would be retained for longer time.

### **3.2.5 Implication for seed dispersal**

These findings have potentially a large impact upon the seed dispersal process. As first, since tortoises remembered the different feature of the food sources, it could be claimed that plants that provide more satisfying fruits, not only would receive more visits from frugivores, but they would also have assured their visits after long periods of time (Di Fiore and Suarez, 2007; Ban *et al.*, 2014). Janmaat *et al.* (2010), for example, observed that mangabeys (*Lophocebus albigena johnstonii*) rely on memory to re-locate fig trees over a period of six years, finding fig trees more efficiently in previously known areas than in newly explored areas. Eighteen months is a time interval longer than the fruiting interval of the majority of plants species in red-footed tortoise habitat (Moskovitz and Bjorndal, 1990), thus tortoises are potentially able to remember the quality of a food source between fruiting periods.

As was mentioned in the introduction (3.2.1), whether fruit removal is the result of random or constantly repeated events, would have different long-term impacts on seed dispersal (see Boyer and López-Corona, 2009). For example, it could be hypothesized that if fruit removal was solely due to an animal that passes randomly through an area and forages from the resources available at the time, the animal would probably forage first from what it perceived as preferred food, then maybe on other neighbouring plants until it gets satiated, because it will not know when it would be the next time it would feed. On the contrary, it could be

hypothesised that if the animal remembers the quality of one or more food sources in the environment it wouldn't probably choose to forage from the less preferred plants of the patch, but it would move to the next preferred food source. Ban *et al.* (2014) found that chimpanzee directed their foraging towards preferred fruiting trees ignoring closer but less preferred fruits. If this is true, the hypothesis mentioned in section 3.1.5, i.e. the location of food sources with respect to other fruiting plants may influence the success of fruit removal (Levey *et al.*, 1984; Fragoso *et al.*, 2003; Hampe *et al.*, 2008), would be still valid for animals that are new to the area or that do not possess good memory.

### **3.3. Experiment 5. Creating prediction with a seed dispersal model: how memories of different quantity and quality of food impact on seed dispersal**

#### **3.3.1 Introduction**

In the previous sections (3.1 and 3.2) it was shown that tortoises were able to learn the association between a colour and different quantity and perceived quality of food, discriminate among them and remember what they have learned for a long period of time (18 months). Then, the possible impacts that these cognitive skills might bring on seed dispersal process were discussed. In this section I present a seed dispersal model which aims to investigate the effects that the cognitive skills tested in the experiment in 3.1 and 3.2 might have on seed dispersal in environment with plants that offers different food quantity (section

3.3.2). different food quality (section 3.3.3) and different quantity and quality combined (section 3.3.4). The model was built in the same way as for the model in chapter 2 (section 2.3) with small changes that are explained in each of the corresponding sections.

### **3.3.2 Quantity model: Hypothesis tested and predictions**

The aim of the present model was to verify how the memory of the animal would affect its choices in an environment where the food sources differ in quantity, and how this, in turn, affects the seed dispersal pattern. In particular the following hypotheses were tested:

H<sub>1</sub>: The interaction between memory and the total quantity of food provided by the plants, predicts the total amount of seeds dispersed in the landscape.

H<sub>2</sub>: The interaction between memory and the difference in quantity of food provided by each of the two plants predicts the amount of seeds dispersed by each plant.

H<sub>3</sub>: The interaction between memory and the quantity value of the plant explains the long distance dispersal of the individual plant.

H<sub>4</sub>: The interaction between memory and the total quantity of food predicts the proportion of landscape travelled by the animals.

H<sub>5</sub>: The interaction between memory and the total quantity of food explains the time of death of the animals.

The predictions were: (1) the total amount of seeds dispersed would depend on the memory of the animal, so that animals with higher memory would disperse more seeds than animals with lower memory. This would be more evident in environments in which the quantity value of the plants is higher, because the food sources are more salient thus easier to be detected by animals. (2) Animals with high memory would be able to remember the quantity value of the plants and choose preferentially the one that provides the biggest amount of food. Thus, the bigger the difference of the quantity values of the plants the bigger would be the inequality in the amount of seeds dispersed from the two plants. (3) If animals with higher memory forage preferentially from the plant with higher quantity value, then they would return to this plant more frequently than to the other plant. This would increase the probability that the seed would be deposited under the high quantity value plant decreasing its long dispersal distance and (4) reducing the proportion of landscape travelled by the animal. (5) Memory would affect the survivorship of the animals, and animal with higher memory would survive longer than animals with low memory because they are better in relocating the best food sources; this would particularly help in environment where plants have low quantity value.

### **3.3.3 Method**

The present model was designed to simulate an environment in which the food sources present different quantities of food. Plants could assume different random starting values in a range between 0 and 1 (0 = no food available; 1= max

quantity of food). The salience of each tree in the landscape is reflected by its value because, as described in the method in chapter 2, section 2, the animal in the model is more attracted by spatial locations with higher values. Therefore, the higher is the value of the plant, the more the animal would be attracted by it. As for the seed dispersal model in chapter 2, two landscapes were simulated for comparison: one with unlimited food sources and one with limited food sources.

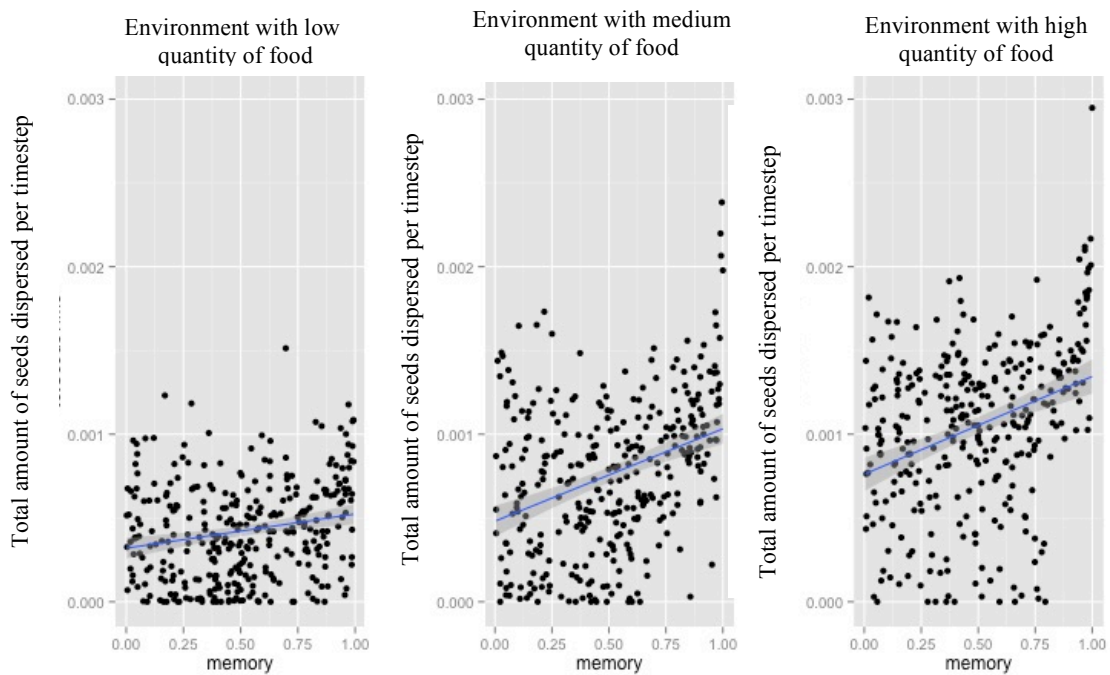
**Table 5 List of the parameters used in the quantity model**

<b>Parameter</b>	<b>Values explored</b>
$\alpha$ : sensory attenuation coefficient	0.5
$\beta$ : memory decay coefficient	Drawn from an exponential logarithm distribution (0.99,1)
$\gamma$ : friction coefficient	0.07
$\kappa$ : satiation coefficient	0.1
$\lambda$ : hunger coefficient	0.99
$\mu_d$ and $\sigma_d$ : parameters of the cumulative lognormal probability density function describing the probability of defecation	4; 0.2
$\mu_s$ and $\sigma_s$ : parameters of the cumulative lognormal probability density function describing the probability of seed excretion	1; 0.2
$T$	10,000
$\kappa_m$ : concentration parameter for von Mises distribution	1.83
<i>Death threshold</i>	0.001
<b><i>Plant Quantity</i>: value of the plant as it appears in the vegetation map</b>	<b>Uniform random numbers in the range [0, 1]</b>
<i>Plant Quality</i> : quality coefficient of the plant	1

### 3.3.4 Results

#### 3.3.4.1 $H_1$ : Total amount of seeds dispersed in the environment

The total amount of seeds dispersed was affected by the interaction between the memory of the animal and the total quantity of food provided in the landscape in environments with unlimited food availability ( $F(1,996)= 14.29$ ,  $p< 0.001$ ): animals with high memory disperse more seeds than animals with low memory, and the amount of seeds dispersed increases with the increase of food availability in the environment (fig.3.5).

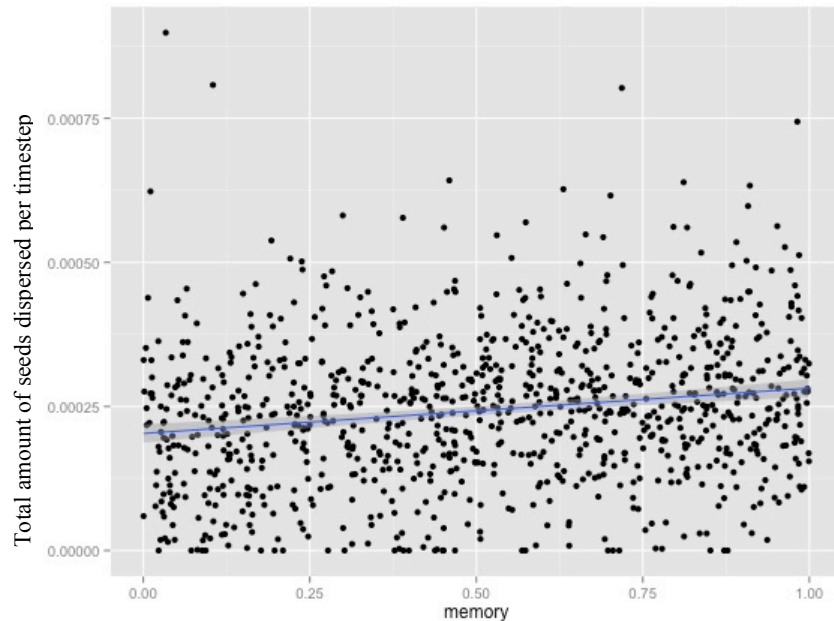


**Figure 3.5** Total amount of seeds dispersed by animals with different memory (0= low memory, 1= high) in environment that provides different total quantity of food. Each point indicates one model iteration (N=1000). The line indicates the average amount of seeds dispersed per timestep  $\pm$  SE.

This interaction loses significance in landscapes in which the food availability is limited ( $F(1,997)= 1.61$ ,  $p= 0.2$ ), but memory remains significant as a main effect ( $F(1, 997)= 79.75$ ,  $p< 0.001$ ). Figure 3.6 shows that animals with higher memory



disperse more seeds than animals with lower memory, but the total amount of seeds dispersed by animals with different memory is similar because the resources are limited.



**Figure 3.6** Total amount of seeds dispersed in environment with limited food availability at different memory (0= low memory, 1= high memory). Each point indicates one model iteration (N=1000). The line indicates the average amount of seeds dispersed per timestep  $\pm$  SE.

#### 3.3.4.2 $H_2$ : individual amount of seeds dispersed from the two plants

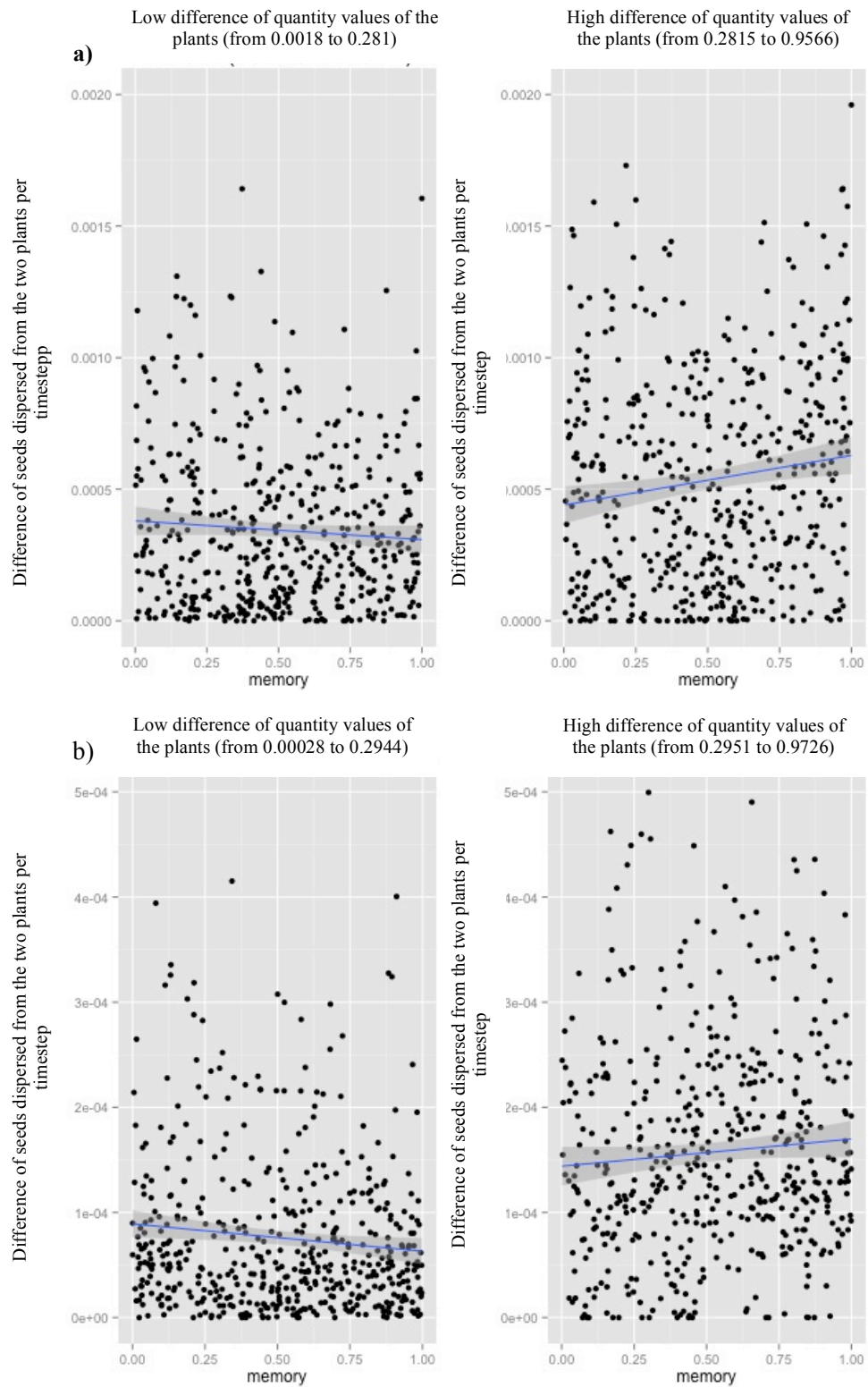
The different amount of seeds dispersed from the two plants was analysed in order to investigate if the plant that with higher quantity value would receive an advantage in terms of amount of seeds dispersed. Results showed that animals with higher memory visited higher value plants more often, creating as a consequence a bigger inequality in the amount of seeds dispersed from the two plants; this interaction between animal memory and the different values of the plants was significant in the landscape with unlimited ( $F(1, 997)= 21.49, p < 0.001$ ) and limited ( $F(1, 997)= 12.54, p < 0.001$ ) food availability. This means that

in environments in which the quantity of food provided by the food sources is highly different, animals with memory are able to take advantage of the most productive tree by foraging preferentially from it and producing a bigger inequality in the amount of seeds dispersed from the trees. When the plants are quantitatively equivalent the preference for one plant disappears and the inequality in the amount of seeds dispersed from the plants tend to be reduced, in particular by animals with high memory. Animals with low memory, on the contrary, seem to behave in the same way no matter the quantitative difference of the plants (fig 3.7a). The same results were obtained in environment with limited food availability (fig. 3.7b).

#### 3.3.4.3 *H<sub>3</sub>: Long distance dispersal*

The long distance dispersal of the plants with the highest and lowest quantity values was analysed. Since animals with high memory choose preferentially the plant with higher quantity values, the expectation was that the seeds of the preferred tree would have shorter dispersal distances than these of the other tree in the landscape, because the animal would spend more time around it. However, the interaction between memory of the animal and quantity value of the plant did not affect the dispersal distance of seeds ( $F(1, 989) = 0.002$ ,  $p = 0.964$ ). Memory was the only factor affecting the dispersal distance of the plants in both type of landscapes (unlimited food availability: memory  $F(1, 989) = 20.43$ ,  $p < 0.001$ ; fruit quantity  $F(1, 989) = 3.75$ ,  $p = 0.053$ ; limited food availability: memory  $F(1, 987) = 8.11$ ,  $p < 0.01$ ; food quantity  $F(1, 989) = 0.33$ ,  $p = 0.562$ )).

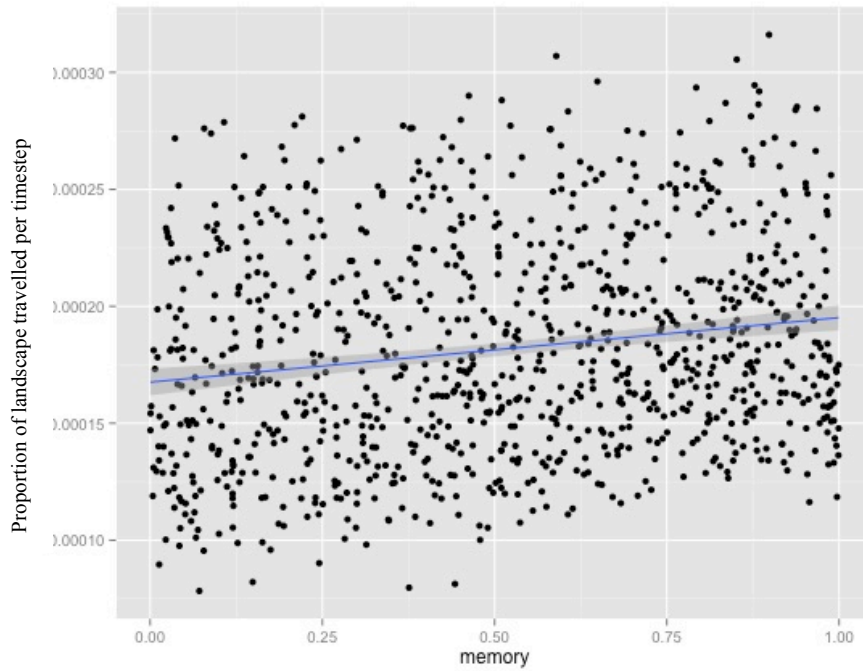
As in the previous model (chapter 2), results showed that the higher the memory of the animal the closer to the parental tree seeds were deposited.



**Figure 3.7** a) Difference of seeds dispersed from the plants in landscape with unlimited food availability at different memory (0= low memory; 1=high memory) and different quantity values of the trees. b) Difference of seeds dispersed from the plants in the landscape with limited food availability at different memory (0= low memory; 1=high memory) and different quantity values of the trees. Each point indicates one model iteration (N=1000). The line indicates the average difference of seeds dispersed from the two plants per timestep  $\pm$  SE.

#### 3.3.4.4 *H<sub>4</sub>: Proportion of landscape travelled*

The proportion of landscape travelled by the animals was not affected by the interaction between memory and the quantity of food provided in the environment ( $F(1, 992) = 0.058, p = 0.808$ ). Animals' movement was influenced exclusively by the distance from each other at which plants were located in landscape with unlimited food availability. In the same way, the interaction was not significant in an environment with limited food availability ( $F(1, 992) = 0.09, p = 0.764$ ), but there, memory influenced the distance travelled as animals with higher cognitive skills travelled more than animals with lower memory ( $F(1, 996) = 33.9, p < 0.001$ ) (fig. 3.8). This might be due to the limited amount of resources that pushed the animals with memory to move more between trees: seed dispersers in the model are more attracted by hexagons of the map with higher values. When the plants are depleted, their map-value decreases, thus the higher value plant, after being consumed, would become the lower value plant. This would make the animals move away from it and prefer the other tree. When part of this tree is consumed, the values of the plants would be exchanged again. This process might make the animals with memory move more. The same would not happen with animals with lower memory because they would forget the values of the plants very quickly, thus their foraging strategy and movement would be unaffected.



**Figure 3.8** Proportion of landscape travelled per timestep in environment with limited food availability at different memory (0= low memory, 1= high memory). Each point indicate one model iteration (N= 1000). The line indicates the average proportion of landscape travelled per timestep  $\pm$  SE.

#### 3.3.4.5 *H<sub>5</sub>: Animals' survival*

Animal survival was affected by the interaction between memory and the total quantity of food provided by the environment, in landscape with unlimited food availability ( $\chi^2(7, N=1000)=24.97, p< 0.001$ ) (fig 3.9). Animals with higher memory survive longer than animals with lower memory and this effect is enhanced in environments that offer a bigger total amount of food, where animals with high memory almost never die (fig 3.9c). This depends on the fact that in environment with higher quantity values, plants (or at least one of them) are more salient than in landscape with low quantity values.

In environments with limited food availability the interaction between animal memory and the total amount of food provided was not significant ( $\chi^2(7,$

$N=1000$ ),  $p= 0.085$ ). The total quantity of food provided in the environment resulted to be the most relevant factor influencing death rate ( $\chi^2(7, N=1000)= 24.97, p< 0.001$ ), so that in an environment with less food available animals die sooner and, as found in the model in chapter 2, memory could actually be a disadvantage ( $\chi^2(7, N=1000)= 119.24, p< 0.01$ ) because the ability to relocate food sources efficiently allows animals to consume the food available more quickly.

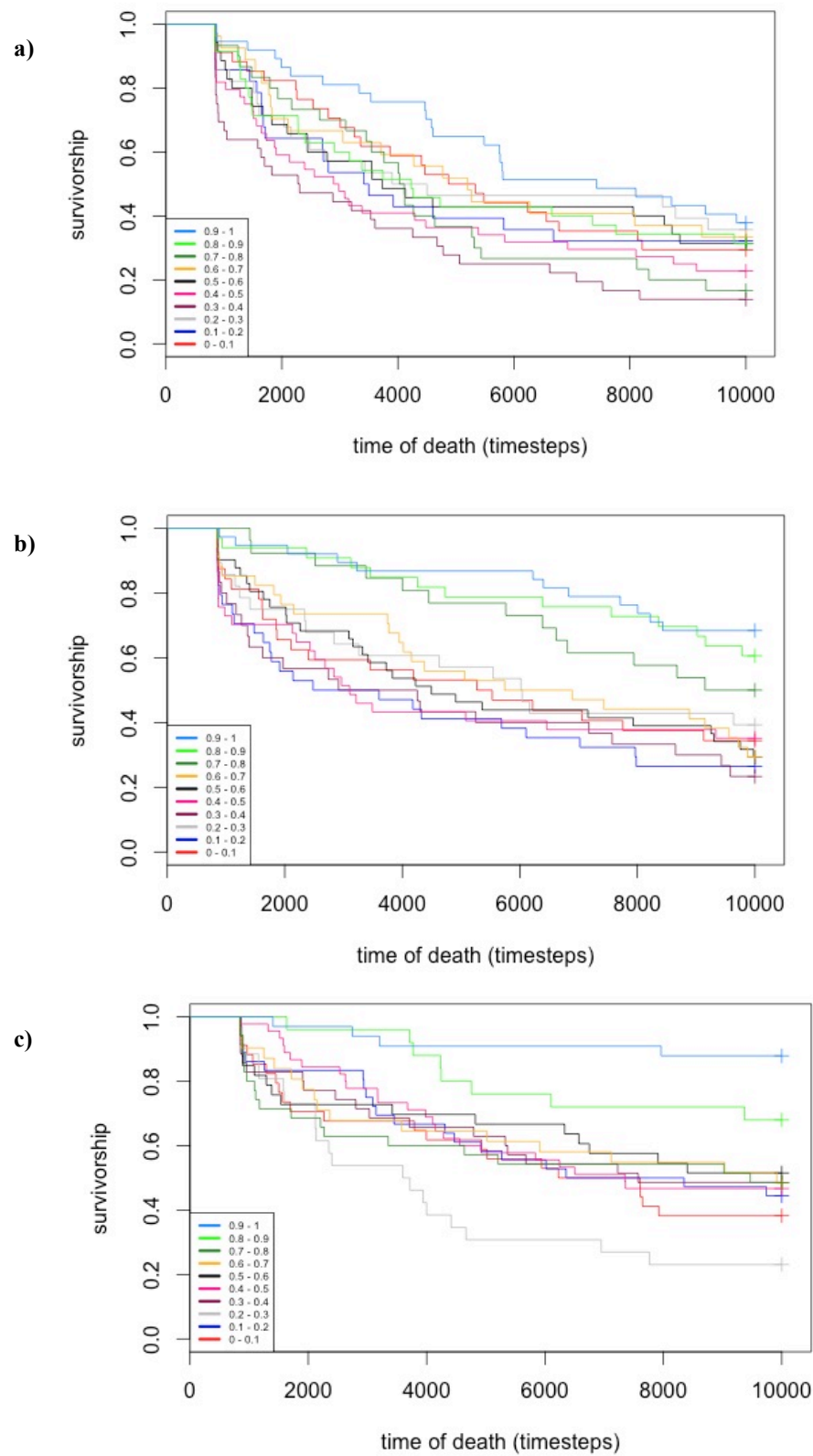


Figure 3.9 Survivorship of animals with different memory in environment with low (a), medium (b) and high (c) total quantity of food. The legend on the bottom-left indicates the range of the values of memory in which the data were grouped exclusively for a clearer representation (0-0.1= low memory; 0.9-1= high memory).

### 3.3.5 Discussion

The present model showed that the quantity of food provided by plants is an important factor in seed removal by frugivores. The amount of seeds dispersed increased when plants in the landscape had higher quantity values. This happened in the model because quantity is a salient feature that could be perceived by the animals, with the result that the higher the quantity value of a plant the more the animals were attracted by it. This is in line with what is found in nature, where the number of frugivores seems to be closely linked with local abundance of fruit (Rey, 1995; Tellería and Pérez-Tris, 2003; Tellería *et al.*, 2008; Gleditsch and Carlo, 2011). Moreover, results support those found in other seed dispersal models (Carlo and Morales, 2008; Boyer and López-Corona, 2009), which analysed the neighbouring effect of plants on seed dispersal. The more attractive the composition of a patch in the environment, the more plants in it would have chances of dispersal (Carlo and Morales, 2008). Thus, as mentioned in 3.1.5, having high food quantity, or a salient feature that attracts animals, could be beneficial not only for the tree itself but also for the neighbouring plants. However, the model presented in this section, showed that these results might be very different depending on the memory skills of the animals: the amount of seeds dispersed in the environment increased exponentially at higher level of memory, showing that the ability to not only perceive, but remember, the location of food sources and base the foraging decision according to previous experiences could increase significantly fruit removal rate. Moreover, memory is the factor that regulates the inequality of dispersal from trees with different quantity values. When the plants have similar values, animals with memory tend to disperse the same amount of seeds from the both plants in the environment (as in the model



presented in chapter 2). On the contrary, when the plants differ in value, animals with memory tend to increase the inequality of seeds dispersed from the trees. This does not happen with animals that have no memory and rely exclusively on their perception.

As also found in the model in chapter 2, high memory provides the plants with shorter seed dispersal than animals with low memory, due to the fact that their movements are driven by the location of food. Carlos and Morales (2008) found that the location of good patches in the environment would affect animals' movements and seed distribution. The present results are in line with these findings, however, once again, these are strengthened by the degree of memory of the animals. The quantity of food available in the environment interacts with memory and, as expected, animals with higher memory survive longer thanks to their ability to relocate food. In landscape with limited food, animals with high memory deplete faster all the resources in the environment, dying sooner than animals with less memory. However, this situation is unlikely to happen in nature, as an animal can normally move to another area once they have consumed the resources in the patch previously used for foraging.

### **3.3.6 Quality model: introduction, hypothesis tested and predictions**

The previous model (3.3.1) showed that whether animals remember the quantity of resource that a plants offer impacts differently on seed dispersal than if they have no knowledge and thus forage at random. Quantity was a salient feature of the plants, thus animals could perceive it. In this section I therefore present the results of a model in which plants present different qualities of food, a non-salient

feature. In a scenario where an inexperienced frugivorous encounters for the first time a fruit, it might not be possible for it to know about the quality of the fruit only by its external features. The animal must eat the fruit first and only then, according to its taste and digestive feedback, the animal will categorise the fruit as good or bad (Sclafani, 1995). As for the previous model with plants of different quantitative value, the aim was to verify how the memory of the animal would affect its choices in an environment where the food sources differ in quality, and how this, in turn, affects the seed dispersal pattern. In particular the following hypothesis were tested:

H<sub>1</sub>: The interaction between memory and total quality of the environment predicts the total amount of seeds dispersed (Total quality value of the environment = sum of the quality of the two plants).

H<sub>2</sub>: The interaction between memory and ratio of the quality of the plants predicts the individual amount of seeds dispersed from the two plants.

H<sub>3</sub>: There is a three way interaction between memory, ratio of the quality of the plants and total quality of the environment that predicts the individual amount of seeds dispersed from the two plants.

H<sub>4</sub>: The interaction between memory and quality of the plant predicts the long distance seed dispersal of the two plants.

H<sub>5</sub>: The interaction between memory and quality of the environment explains the proportion of landscape travelled by the animal.

H<sub>6</sub>: The interaction between memory and quality of the environment explains the survivorship of the animals.

The predictions are: (1) there is an interaction between memory and total quality of the environment so that the total amount of seeds dispersed would depend on the memory of the animal (animals with higher memory would disperse more seeds than animals with lower memory); this would be more evident in environment in which the total quality is low, because, there, animals would have to forage more times because they receive fewer calories for each foraging bouts than in environments where the plants have higher quality values. More foraging bouts equals to more seeds ingested and dispersed. Thus, in lower quality environments the amount of seeds dispersed would be bigger than in environment with high quality value. (2) Animals with high memory would be able to remember the quality of the plants and choose preferentially the one that provides better quality food. Thus, the lower the ratio of the quality of the plants (the more they differ in quality) the bigger will be the difference in the individual amount of seeds dispersed from the two plants. (3) Animals with more memory would forage preferentially from the plant with higher quality values, therefore they would return to this plant more frequently than to the other plant, as a consequence the probability that the seed would be deposited under the high quality plant would increase, decreasing its long distance dispersal. (4) The proportion of landscape travelled should be higher for animals with high memory in environments where the total quality is higher. As mentioned above, when the resources have better quality values the animal should forage less frequently, because it receives more “calories” when eating, thus it would have more time to move far from the plant and explore more of the landscape. (5) Animals would survive longer if they have more memory, because they are better at relocating plants; this would be particularly helpful in low quality environments.

### 3.3.7 Method

The model simulated an environment in which the food sources are qualitatively different. In the model, plants assume different random quality values in a range between 0 and 1, where 0 represents null quality and 1 maximum quality. The quality value of the plant cannot be perceived (in advance of eating) by the animal, but it can be remembered: both plants in the environment are perceived in the same way as they both have a quantitative value of 1 (value of the hexagons containing the plants); when the animal eats for the first time, it would associate to the plant which it is feeding from a quality value of 1 (maximum quality). This is because the animal considers the first tree as the best in the environment, as it is the only one the animal has sampled, and it has no previous experience of other types of food. When the animal finds and forages from the second plant, the quality values are then re-assessed: the tree with the higher quality value would assume a value of 1, while the second tree would assume a value equal to the ratio of the two values (minimum quality value divided by the maximum quality value). In this way, the values of the plants are relative to each other. For example, if one plant in the environment has a quality of 0.2, when the animal forages from this for the first time, it would perceive the plant to have a value of 1. Once the forager finds the second plant with a quality value of 0.8, this new plant would now assume a coefficient of 1, while the previous tree gains a coefficient of 0.25 ( $\text{min quality} / \text{max quality} = 0.2/0.8$ ). The quality coefficients are multiplied with the values in the memory map corresponding to the location of the plants, so that the memory of the tree of higher quality would remain the same (because plants with higher quality have a coefficient of one), while the memory of the second plant will be reduced. The

quality of the plant affects the state of the animal, so that every time that the animal feeds, the amount of food eaten is multiplied by the quality value of the tree that it is foraging from.

As for the previous model, two landscapes were simulated for comparison: one with unlimited food sources and one with limited food sources.

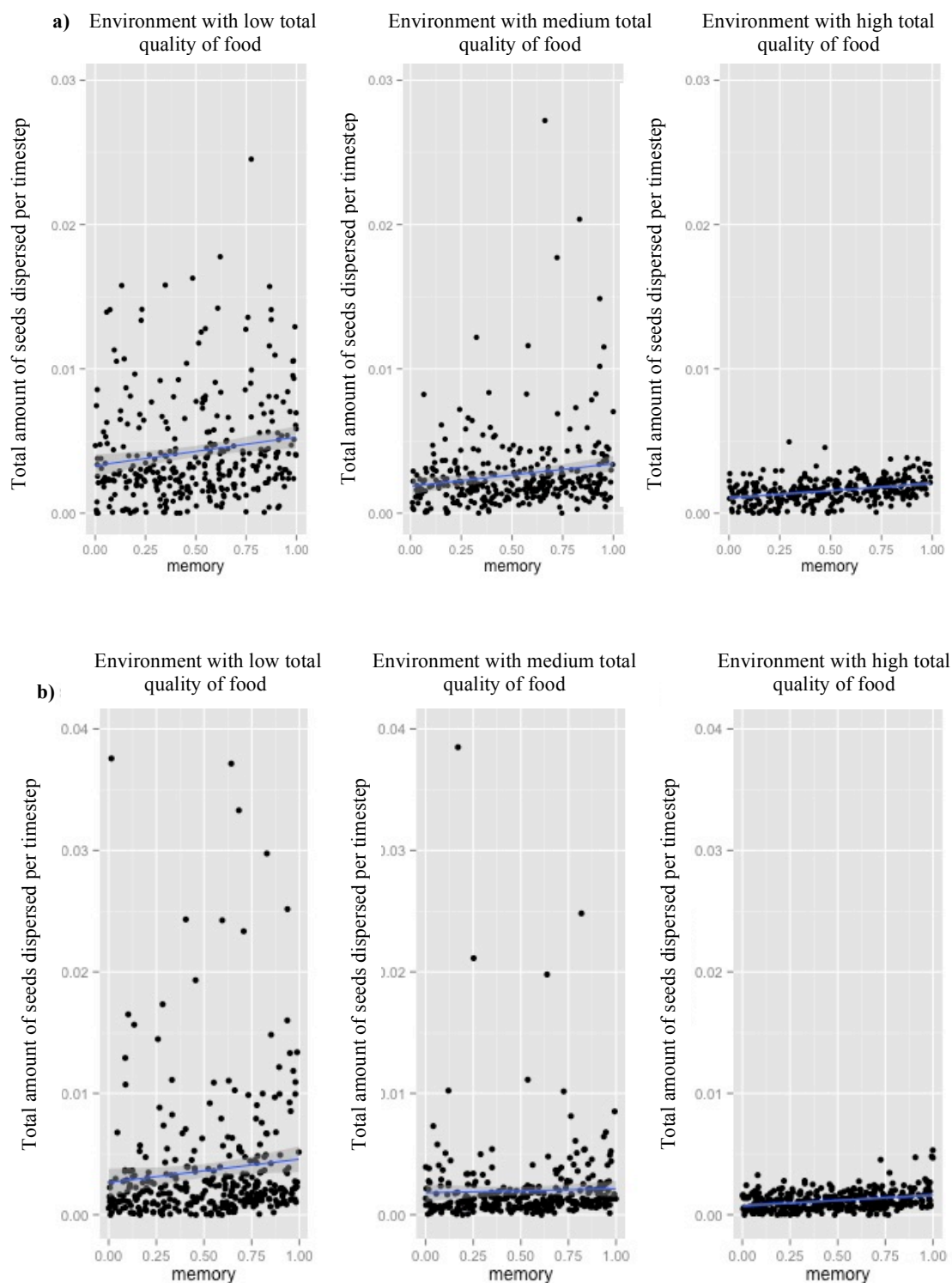
**Table 6 List of the parameters used in the quality model**

Parameter	Values explored
$\alpha$ : sensory attenuation coefficient	0.5
$\beta$ : memory decay coefficient	Drawn from an exponential logarithm distribution (0.99,1)
$\gamma$ : friction coefficient	0.07
$\kappa$ : satiation coefficient	0.1
$\lambda$ : hunger coefficient	0.99
$\mu_d$ and $\sigma_d$ : parameters of the cumulative lognormal probability density function describing the probability of defecation	4; 0.2
$\mu_s$ and $\sigma_s$ : parameters of the cumulative lognormal probability density function describing the probability of seed excretion	1; 0.2
$T$	10,000
$\kappa_m$ : concentration parameter for von Mises distribution	1.83
<i>Death threshold</i>	0.001
<i>Plant Quantity</i> : value of the plant as it appears in the vegetation map	1
<b><i>Plant Quality</i>: quality coefficient of the plant</b>	<b>Uniform random numbers in the range [0, 1]</b>

### 3.3.8 Results

#### 3.3.8.1 $H_1$ : Total amount of seeds dispersed in the environment

The total amount of seeds dispersed in the environment was affected by the interaction between memory of the animal and the total quality of the environment in landscape with unlimited ( $F(1,996)=12.88, p<0.001$ ) and limited ( $F(1,996)=7.02, p<0.01$ ) food availability. Animals with higher memory dispersed more seeds than animals with lower memory, in particular in environments with a low total quality of food. This is due to the fact that, in low quality environments, the animals are less satiated every time they feed, therefore they have to forage more than they do in a comparison with high quality environment. As a consequence they ingest and disperse more seeds (fig. 3.10).

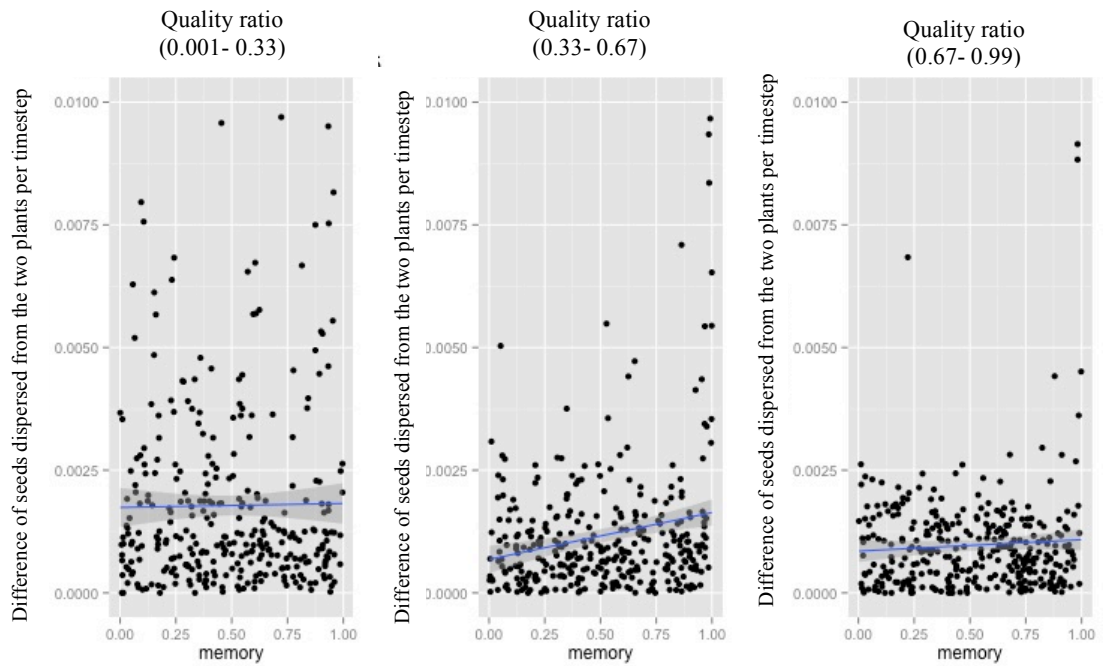


**Figure 3.10** a) Total amount of seeds dispersed at different memory (0= low memory, 1= high memory) in environment that provides different total quality of food and unlimited resources. b) Total amount of seeds dispersed at different memory (0= low memory, 1= high memory) in environment that provides different total quality of food and limited resources. Each point indicates one model iteration (N= 1000). The line indicates the average amount of seeds dispersed per timestep  $\pm$  SE.

### 3.3.8.2 $H_2$ : *Different amount of seeds dispersed from each of the plants*

The different amount of seeds dispersed from the two plants was analysed in order to understand if the plant that invested in higher quality food, would receive in exchange an individual advantage in seed dispersal terms. This was the case, and enhanced by the memory of the animals: the difference of seeds dispersed from the two plants was affected by the interaction between the memory of the seed disperser and the ratio between the quality values of the plants ( $F(1,995)=9.48$ ,  $p < 0.01$ ; fig 3.11) in an environment with unlimited food availability. This means that animals with high memory remember which plant offers the best quality of food and forage preferentially from it. Figure 3.11 shows that this interaction seems to acquire more importance when the ratio of the quality is between 0.33 and 0.67, thus when a plant has a quality value, which is approximately the double of the other. When the quality ratio is low (big difference in plants' values) the inequality in the individual amount of seeds dispersed is the same at every memory. This might happen because, when plants are very different from each other, the higher number of time the animals have to forage could mask the effect of memory. When the ratio is high, thus plants are similar in value, animals with memory tend to feed from both plant equally (as seen in model in chapter 2).





**Figure 3.11** Difference of seeds dispersed from the plants in landscape with unlimited food availability at different memory (0= low memory; 1=high memory) and different quality ratio of the trees. Each point indicates one model iteration (N= 1000). The line indicates the average difference of seeds dispersed from the two plants per timestep  $\pm$  SE.

In an environment with limited food availability the above interaction is no longer significant ( $F(1,995)= 2.50$ ,  $p= 0.114$ ). This happens because once the animals have depleted all the food provided by one plant, they are forced to forage from the other.

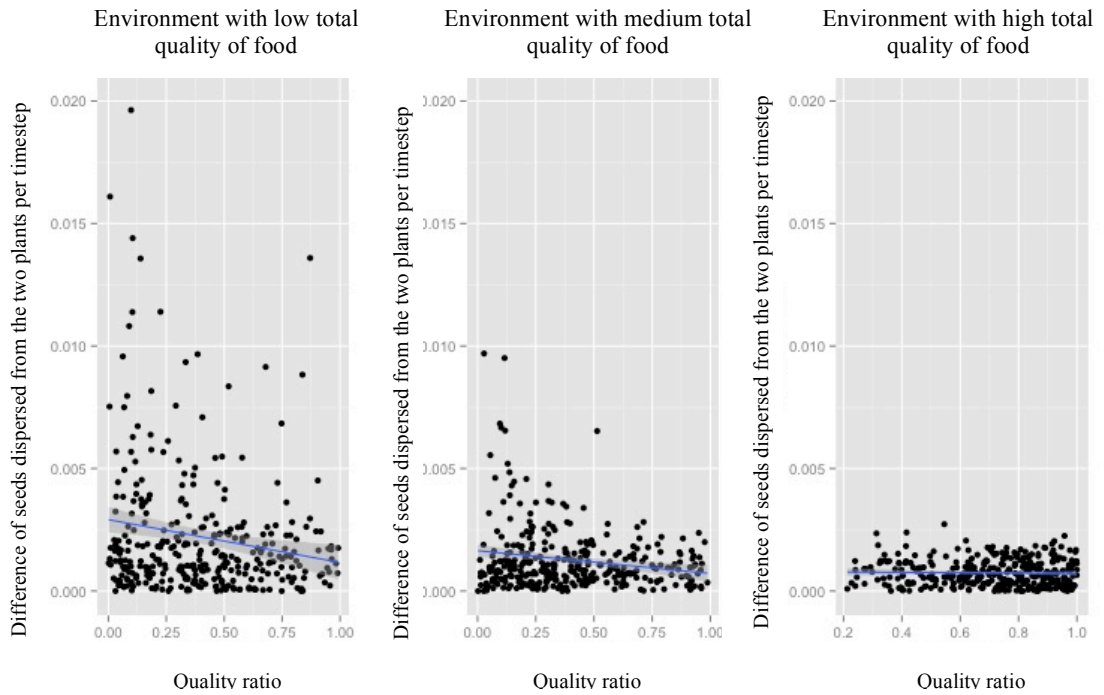
### 3.3.8.3 $H_3$ : Inequality of seed removal in environments of different qualities

From the results obtained until now, it looks that the amount of seeds dispersed is bigger in poor quality environment (with low total quality) because the animals are forced to forage more frequently, and for the plants that provides the best quality of fruits. A question that arises was if in high quality environment

the individual quality of the tree matters in animal choice of food source. Thus, the three way interaction between the total quality of the environment, animal memory and the ratio of the plants' quality values was examined. This was significant ( $F(1,991)=32.99, p< 0.001$ ), because in low quality environments the individual quality of the plant is essential in determining animal food choice: when plants present very different quality values the difference of seeds dispersed is higher, than if the plants have similar value (high ratio). On the contrary, in high quality environments the individual value of the trees loses importance and seeds are dispersed evenly from both trees (fig.3.12). This is enhanced at higher memory. The same results were found in the landscape with limited food availability (interaction:  $F(1,984)=13.48, p< 0.001$ ).

#### 3.3.8.4 *H<sub>4</sub>: Long distance dispersal*

The long dispersal distances of seeds of the plants with the highest and lowest quality values were analysed. The same prediction as for the quantity model was made, that the preferred tree would suffer shorter dispersal distance because the animals would spend more time around it. However, long dispersal distance was not affected by the interaction between memory and the quality value of the plants ( $F(1,1843)= 1.75, p=0.19$ ). Memory ( $F(1,1843)= 44.10, p< 0.001$ ) and distance between the plants ( $F(1, 1843)=21.96, p< 0.001$ ) were the two main effects that influenced the dispersal distance of seeds from the parental tree. As in the previous model in chapter 2, the higher the memory, the shorter the distance from the parental plant at which the seeds were deposited. The same was found in landscape with limited food availability ( $F(1, 1843)= 18.12, p< 0.001$ ).



**Figure 3.12** Difference of seeds dispersed from the two plants according to the quality ratio of the trees in environment with low, medium and high total quality. Each point indicates one model iteration (N=1000). The line indicates the average difference of seeds dispersed from the two plants per timestep  $\pm$  SE.

### 3.3.8.5 $H_5$ : Proportion of landscape travelled

The proportion of landscape travelled by the animals was not affected by the interaction between memory and the total quality of the environment (unlimited food:  $F(1,992)=1.22$ ,  $p=0.27$ ; limited food:  $F(1,992)=1.59$ ,  $p<0.207$ ). The landscape travelled was affected exclusively by the distance between the plants and this was verified in landscapes with unlimited ( $F(1,992)=45.33$ ,  $p<0.001$ ) and limited ( $F(1,992)=3.62$ ,  $p=0.05$ ) food availability.

#### 3.3.8.6 *H<sub>6</sub>: Animals' survival*

The death rate of the animals was not affected by the interaction between animal memory and the total quality of the environment in landscape with unlimited food availability ( $\chi^2(1, N= 1000)= 1.54, p< 0.123$ ). Memory as a main effect influenced the survivorship of the seed dispersers ( $\chi^2(1, N= 1000)= 97.44, p< 0.01$ ), and, as expected, animals with higher cognitive skills were able to survive longer than animals with lower cognitive skills. Memory affected the death rate of animals in landscape with limited food resources as well ( $\chi^2(1, N= 1000)= 26.53, p< 0.001$ ) but as in the previous models, in limited environments, animals with higher memory died at similar rates to animals with low memory.

#### 3.3.9 Discussion

As for the previous model (3.3.2), results of the present model confirmed predictions by showing that the quality of the food sources is an important feature to attract frugivores and interacts with animals' memory affecting the total amount of seeds dispersed in the environment, the inequality of seeds removal from the two plants and the rate of survivorship of the animals. As mentioned for the model on quantity, these results support the finding of Carlos and Morales (2008), according to which the features and aggregation of plants would influence seed removal patterns, adding the information that this effect might be enhanced by the memory of seed dispersers. In particular, in the present model the quality of fruits was not a salient feature, because the animals couldn't perceive it, but only remember it after foraging from the plant. Thus, in this case, memory can structure the outcome of seed dispersal favouring the dispersal of the better food

source. For example, chimpanzees in the wild seem able to remember the location of preferred trees even when these are outside their perception field (Ban *et al.*, 2014) avoiding low quality food sources even if they are closer (Glander, 1978). This leads to an increase of seed dispersal of plants that offer fruits of better quality.

The model also suggests that the total quality of the plants in the environment affects the removal rate. If plants provide highly energetic resources, the animals forage fewer times, ingesting in consequence fewer seeds, because they are satiated by a smaller amount of fruits. On the contrary if plants in the landscape are low in quality, the animal has to forage more times in order to get satiated, ingesting and dispersing a bigger amount of seeds. To compensate for the reduced foraging in high quality environments, a strategy could be to increase the amount of seeds provided in high quality fruit, so that even if the number of fruits eaten is lower, the amount of seeds ingested and dispersed will remain high. However, to my knowledge, no studies investigate the existence of a trade-off between calories provided and quantity of seeds in a fruit. Studies focus mainly on the trade off between size of seeds-number of seeds and relate these features to the co-evolutionary pressure from the main seed disperser (Levey, 1987; Parciak, 2002), or to the success of establishment after deposition (Schupp, 1995; Turnbull *et al.*, 1999).

As in the previous models (section 3.3.2 and chapter 2) the long dispersal distance of seeds depended exclusively on animals' memory, regardless of the quality of the plant. Thus, once again the model underlines how the ability of

animals to remember the location of the food sources plays a fundamental role in the outcome of seed dispersal.

### **3.3.10. Quantity and Quality model: Introduction, hypothesis tested and predictions**

In the previous models, the effects of animals with different memory in environments with different quantity or different quality of food were investigated separately. The present model combined these two features together. The aim of the present model was to investigate how the memory of the animal would affect its choices in environments where the food sources differs in quality and quantity, and how this, in turn, affects seed dispersal. The following hypothesis were tested:

H<sub>1</sub>: The three way interaction between memory, quality of the environment and quantity provided in the environment predicts the total amount of seeds dispersed in the landscape (quality of the environment = sum of the quality of the two plants; quantity of the environment = sum of the quantity of the two plants)

H<sub>2</sub>: The three way interaction between memory, ratio of the quality value of plants and difference of quantity values of plants explains the inequality of seeds dispersed from the two plants.

The predictions are: (1) as in the previous model, memory plays an important role in the amount of seeds dispersed. However, this would be lower in environments that offer higher quality of food because, in these landscapes, the animal can forage less as it receives a bigger amount of “calories” at every foraging bout and the resources are less salient (low quantity value) because they would be more difficult to be detected by the animals. (2) Animals with high memory would increase the inequality of the seeds dispersed from the two plants in the landscape when the ratio of the quality is lower (the plants are very different in quality) and the difference of the quantity of the plants is bigger.

### 3.3.11 Method

The method was the same as in chapter 2, with the difference that both the quality and quantity of plants were assigned a random value as described in sections 3.3.3 and 3.3.7. The model was run for 10 000 iteration, instead of the usual 1000, to better identify the possible interactions between parameters.

**Table 7 List of the parameters used in the quantity-quality model**

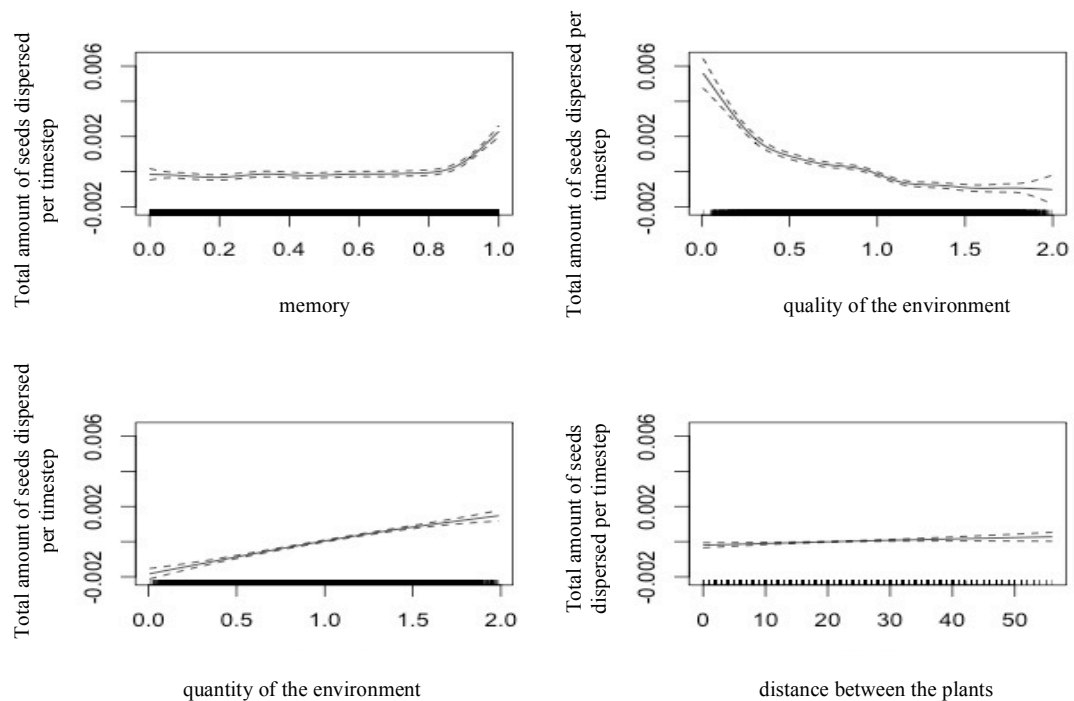
Parameter	Values explored
$\alpha$ : sensory attenuation coefficient	0.5
$\beta$ : memory decay coefficient	Drawn from an exponential logarithm distribution (0.99,1)
$\gamma$ : friction coefficient	0.07
$\kappa$ : satiation coefficient	0.1
$\lambda$ : hunger coefficient	0.99
$\mu_d$ and $\sigma_d$ : parameters of the cumulative lognormal probability density function describing the probability of defecation	4; 0.2
$\mu_s$ and $\sigma_s$ : parameters of the cumulative lognormal probability density function describing the probability of seed excretion	1; 0.2

$T$	10,000
$\kappa_m$ : concentration parameter for von Mises distribution	1.83
<i>Death threshold</i>	0.001
<b><i>Plant Quantity</i>: value of the plant as it appears in the vegetation map</b>	<b>Uniform random numbers in the range [0, 1]</b>
<b><i>Plant Quality</i>: quality coefficient of the plant</b>	<b>Uniform random numbers in the range [0, 1]</b>

### 3.3.12 Results

#### 3.3.12.1 $H_1$ : Total amount of seeds dispersed in environment

The relationship between the total amount of seeds dispersed in the environment and the predictors as main effects were observed (fig. 3.13). The effect of animal memory and the sum of plant qualities were better explained by a quadratic curve, thus a quadratic term was used in the statistical model.



**Figure 3.13** Relation between the total amount of seeds dispersed in the environment per timestep and the predictors.



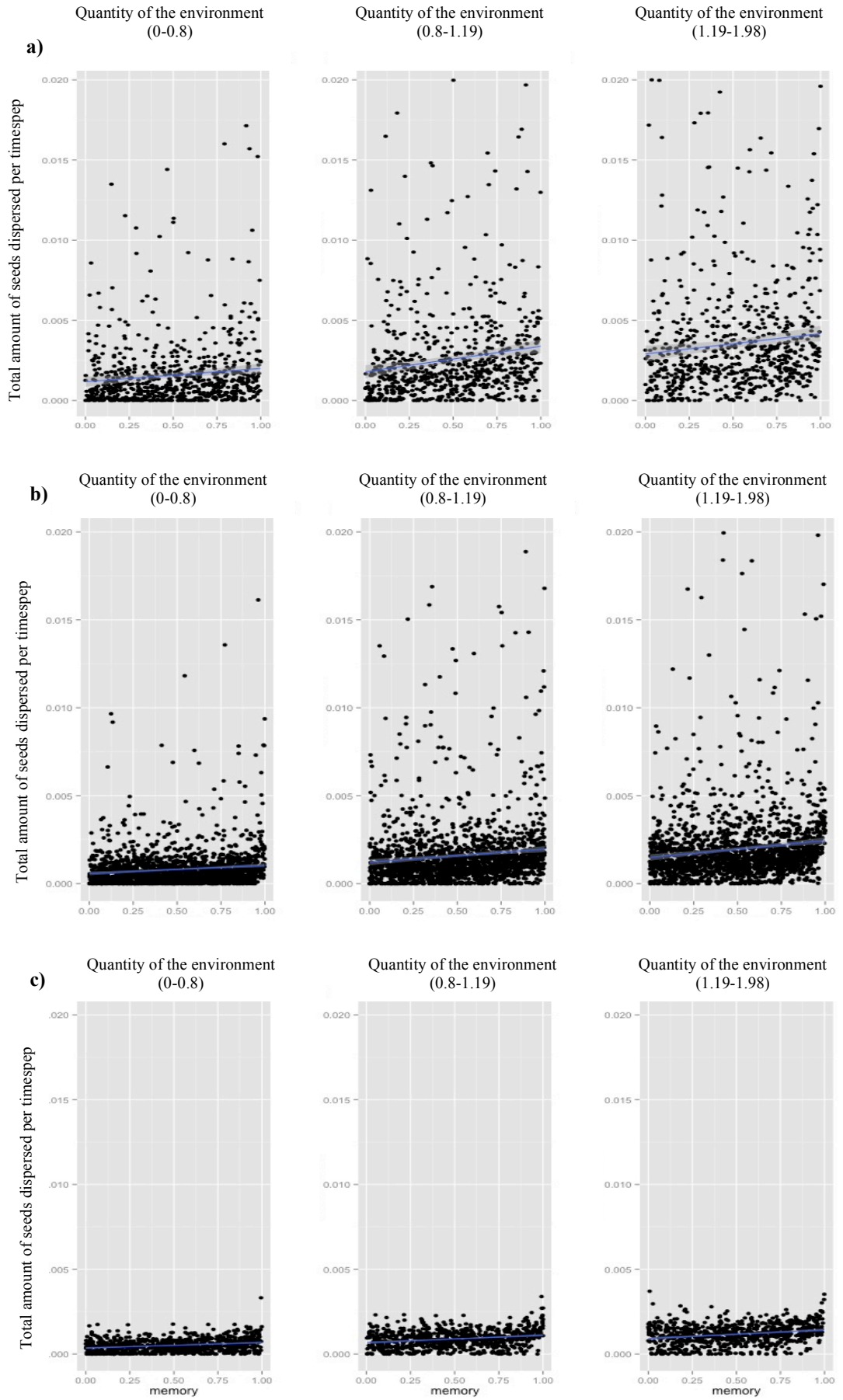
As expected, the interaction between memory and the total quantity and quality of food provided in the environment affected the total amount of seeds dispersed in the environment ( $F(1, 9991) = 5.24, p = 0.02$ ). Animals with high memory dispersed more seeds than animals with low memory and the total amount of seeds that they dispersed was higher in environments in which the plants had lower quality of food and the resources were more salient (higher quantity value) (fig 3.14). The same interaction was found to be significant in an environment where the food availability was limited ( $F(1, 9984) = 44.23, p < 0.001$ ). Data in either landscape followed the same trend, thus, to avoid redundancy, figure 3.14 shows only the data from environment with unlimited food availability.

### 3.3.12.2 $H_2$ : Amount of seeds dispersed from the two plants

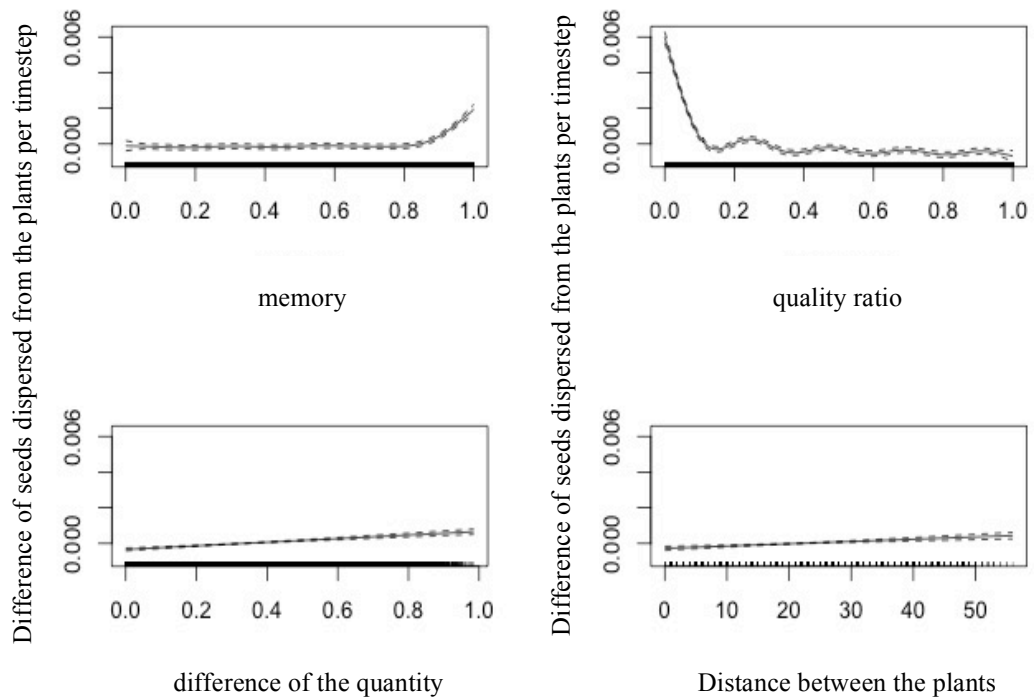
Once again, the relations between the difference of seeds dispersed from the two plants and the explanatory variables tested were observed (fig. 3.15). Animals' memory and ratio between the qualities of the plants were entered into the model as quadratic terms.

Opposite to the predictions, the amount of seeds dispersed from the plants was not affected by the interaction between memory, difference between the quantity of the plants and different ratio of the plants in landscape with unlimited food availability ( $F(1, 9988) = 2.08, p < 0.149$ ). The difference of seeds dispersed from the two plants was influenced by the interaction between memory and the ratio between plants' quality ( $F(1, 9988) = 70.71, p < 0.001$ ) as found in the previous model investigating quality. The interaction between memory and difference quantity of the plants, was not significant ( $F(1, 9989) = 3.5, p = 0.06$ ). This means

that animals with memory are able to remember the information on the quality of the plants and use this more than the salience of the tree to decide which food sources to forage from.

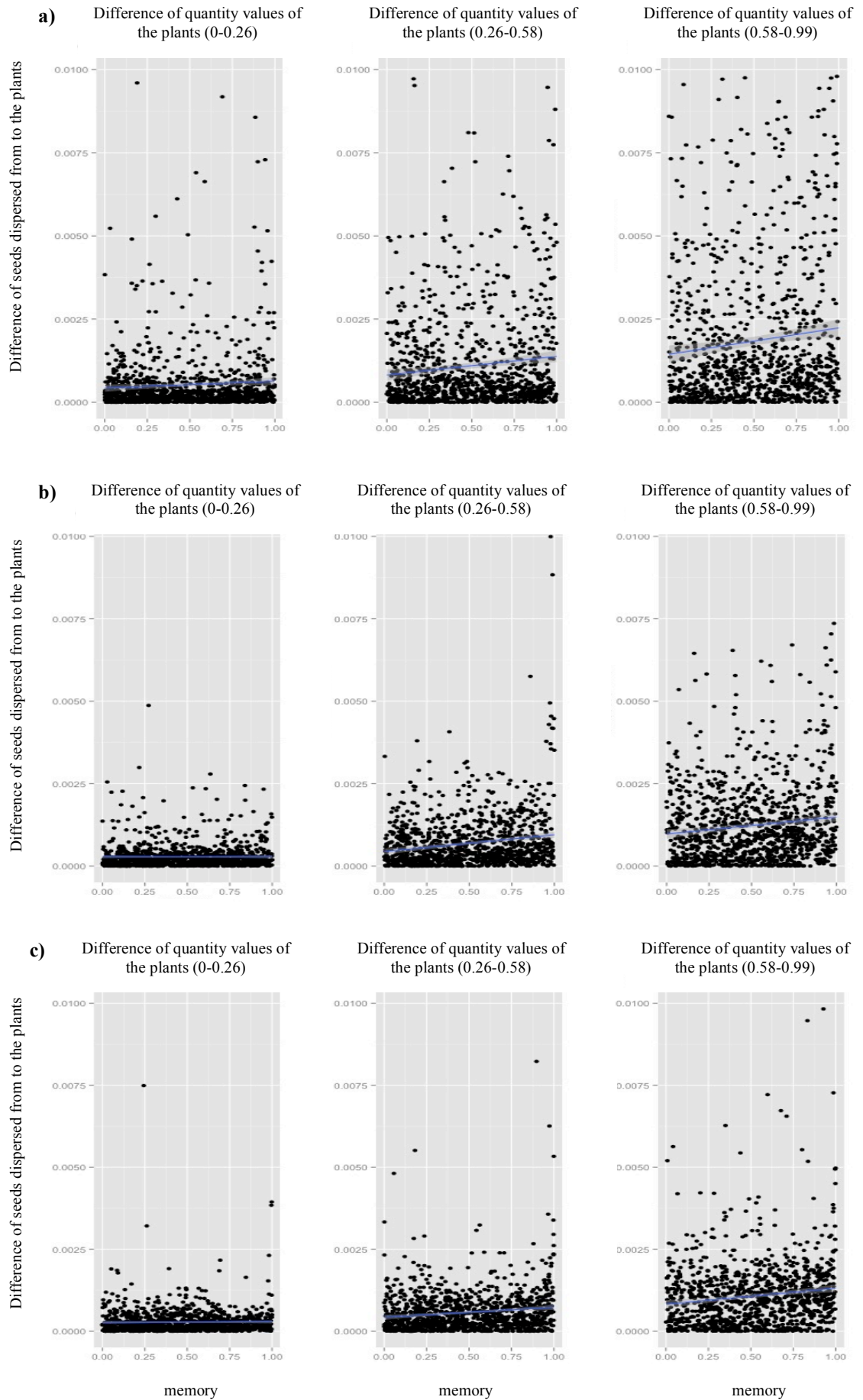


**Figure 3.14.** Total amount of seeds dispersed at different memory (0= low memory, 1=high memory), different quantity and different quality of the environment. (a) quality value= 0-0.66; (b) quality value= 0.66-1.32; (c) quality value= 1.32- 1.99. Each point indicates one model iteration (N= 10000). The line indicates the average amount of seeds dispersed per timestep  $\pm$  SE.



**Figure 3.15 Relation between the difference of seeds dispersed from the plants per timestep in the environment and the predictors.**

In environments in which the food availability is limited, where plants could be depleted, the aforementioned three way interaction affected the amount of seeds dispersed from the two plants ( $F(1, 9984) = 13.78, p < 0.001$ ). Seeds are dispersed more unevenly in environments where the food sources differ in quantity and quality, and this is amplified by animals' memory. Figure 3.16 shows that at higher memory the difference of seeds dispersed increase. This increase is more evident when the difference of the quantity values of the plants is higher. Moreover, the difference of the seeds dispersed from the plants, is higher at lower ratio of the quality of the plants (fig. 3.16a).



**Figure 3.16.** Difference of seeds dispersed from the plants at different memory (0=low memory, 1=high memory), different quantity and quality ratio of the trees: (a) quality ratio= 0- 0.33;(b) quality ratio= 0.33- 0.66;(c) quality ratio=0.66- 0.99; in landscape with limited food availability. Each point indicates one model iteration (N=10000). The line indicates the average difference of seeds dispersed per timestep  $\pm$  SE.

### **3.3.13 Discussion**

This last model showed once again how memory is important in the seed dispersal outcome of plants with different quality and quantity of food. In particular, it showed how both the availability of resources and memory impacts upon the total amount of seed dispersed. Results showed that in environment with low quality plants, a salient feature like quantity of food is essential to increase the fruit removal rate. While in environment high in quality, the quantity of fruit provided is less relevant because the animal get satiated with a smaller amount of food. This is supported by the fact that the inequality of the seed dispersed from the two plants depended exclusively on the interaction between memory and the quality of the tree. This means that, potentially, if animals are able to remember the quality of a fruit this would be more important than the salience of the fruit itself.

## **3.4 General discussion**

In this chapter it was explained how an animals' motivation toward certain types of food, and in particular the ability to remember about features of food sources previously visited, leads to an increase in seed dispersal of the preferred plant. Experiment 2 showed how tortoises were able to discriminate between quantity and perceived quality of food by using visual cues and showed a bigger motivation for larger amounts of food rather than preferred ones. To follow, experiment 3 showed the ability of red-footed tortoise to remember the visual task previously learned after a period of 18 months. This simple experiment showed

how even after one year and a half the motivation for the preferred food remained unaltered. The information collected in experiments 2 and 3 were subsequently used to create a model with the aim of investigating how memory would impact seed removal and dispersal from plants of different quantity and quality of fruit. The biggest finding was that the memory skills of the animals are actually what impact the most in the seed dispersal process: the model showed that only the preference for a certain type of food would result in a much less efficient seed removal if the animal would not possess a good memory to remember about the best food sources (Corlett, 2011). Thus, the repetition of an event, e.g. foraging multiple times from certain patch instead of others, might be one of the process that constitute an advantage for a plant in terms seed removal. This is in line with the results found by Boyer and López-Corona (2009). Carlos and Morales (2008) and Aparicio *et al.* (2013), suggested that the rate of seed removal depends in particular on the number of frugivores present in a certain area. This is certainly true, but results of the model presented in this chapter suggest that plants in an environment with fewer seed dispersers equipped with high memory skills might receive the same fruit removal services as if they were in environment with a larger number of frugivores with lower memory skills. Memory also contributes to the animals' survival, as it allows them to locate more efficiently food sources in the environment thereby avoiding starvation. Therefore, although long-term memory has been proven to represent a cost for the animals (Merry and Kawecki, 2005), results of this chapter suggest it is a cost worth taking, as it enhances animals foraging ability, increases their survivorship and decreases the costs associated with the searching for good food sources. The empirical experiment supports this, as it showed that tortoises remember exactly the features indicative

of the quantity and quality of food after 18 months, thus suggesting that frugivores do invest in this kind of memory.

However, from the plants' point of view, as seen also in the model in chapter 2, memory brings also disadvantages in terms of long dispersal distance, as animals with higher memory tend to disperse seeds closer to the parental plant where the mortality is potentially higher (Janzen, 1970), and reducing the possibility to colonise new habitat (Howe and Smallwood, 1982). Thus, it looks that the best strategy for a plant would be to attract as many different seed dispersers as possible, in terms of behaviour and cognitive skills, in order to receive different benefits from all of them. This is probably the reason why plants invest so much in colourful signals and scent of fruits (Schaefer *et al.*, 2004; Cazetta *et al.*, 2009) as the salience of a fruit in the environment remains the best way for plants to be noticed by multiple dispersers (Schaefer *et al.*, 2004).



# CHAPTER 4

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## 4. WHEN?

### 4.1 Experiment 6: food anticipatory behaviour in the red-footed tortoise

#### 4.1.1 Introduction

In the general introduction (chapter 1, section 1.4.6-1.4.7), it has been introduced the debate about how animals might perceive the passage of time and how they might use this skill to anticipate future events. Moreover, how anticipatory skills might lead to an increase of seed dispersal has been briefly presented.

As mentioned, the ability to time and anticipate predictable and stable events might be particularly important for animals that forage on food sources that are predictable in time and space, such as these available to nectarivores and frugivores (Henderson *et al.*, 2006). Being able, for example, to predict when a tree would produce fruit and for how long these would be on the plant would be a great advantage for the animal; it would be able, in fact, to direct its foraging towards productive food sources, reducing the cost associated to the visits of non-

productive sites (Wilkie *et al.*, 1996). Hummingbirds (*Selasphorus rufus*), for instance, are able to remember the refill time of nectar in different flowers (Henderson *et al.*, 2006): artificial flowers of two different colours were created; one refilled with a sucrose solution every 10 minutes, while the other every 20 minutes. Hummingbirds visited the '10 minute' flowers more often than the '20 minute' flowers, and the visits approximately matched the refill time. The authors suggested that hummingbirds perceived the amount of time passed after their last visit to the flowers, thereby avoiding non-rewarded food sources (Henderson *et al.*, 2006). Such a capability seems to be spread among animals (see section 1.4.7), as honey bees (*Apis mellifera*) have been observed to schedule their foraging flights in anticipation of the time of the day in which the concentration of nectars was at its peak (Corbet and Delfosse, 1984) so as pigeons, mice and monkeys were observed to gather in places where the food was regularly provided just before the food delivery time (Daan and Koene, 1981; Biebach *et al.*, 1989; Wilkie *et al.*, 1996; Waite *et al.* 2001; Roberts, 2002; Mistlberger, 2009)

The lack of fruit – frugivorous specialization causes high competition among frugivores species (Gautier-Hion *et al.*, 1985). A major overlap in the fruit diet of frugivores was in fact found in both close and distant related taxa (Fleming, 1979; Gautier-Hion *et al.*, 1985). Therefore, the ability to anticipate the fruiting period might allow animals to get to the plant first. This would increase their chances of actually finding food and/or getting priority access in environments where the food is limited. In particular, the replacement of harvested fruit is about every one-two days (Howe and Vande Kerckhove, 1979; Chapman *et al.*, 2005), thus it is essential to know whether frugivores are prompted by their own circadian oscillators to revisit fruiting trees every 24 hour

cycle. If this is the case, the plant would have assured seed dispersers during its fruiting period. However, to my knowledge, no studies have investigated anticipatory abilities in frugivores other than primates and birds (Waite *et al.*, 2001; Feeney *et al.*, 2011), despite other animals being important seed dispersers, such as reptiles. Many studies have investigated the physiological mechanisms behind circadian systems of reptiles, e.g. pineal complex, retina and lateral eye (for a review see Tosini *et al.*, 2001), however, none of these explored whether reptiles use their circadian ability to anticipate events. Therefore, the first aim of the present experiment was to verify whether the frugivorous red-footed tortoise is able to anticipate food delivery on a 24 hour cycle, and whether it expresses it in the same way as mammals, i.e. increasing its activity around one hour prior the food delivery time (see introduction, section 1.5.5). The prediction was that, after a testing period, during which the food was delivered at fixed time, if tortoises had learned to anticipate the food delivery time, a significant interaction between tortoises activity in the first and last days of testing should be found. This would indicate that activity of tortoises increased before their food delivery time in the last testing days in comparison with the first ones, when they were not yet familiar with the task.

However, plants do not produce fruit continuously, thus once the fruiting period has ended, it is important for animals to stop returning to the same tree, as it is not productive anymore, and search in the environment for more profitable food sources. This means that frugivores must extinguish their circadian anticipatory activity rapidly once the reward is no longer provided in order to switch quickly to more favourable resources (Saunders, 2002; Tautz, 2008; Moore *et al.*, 2011). It was hypothesised that a rapid extinction of the circadian

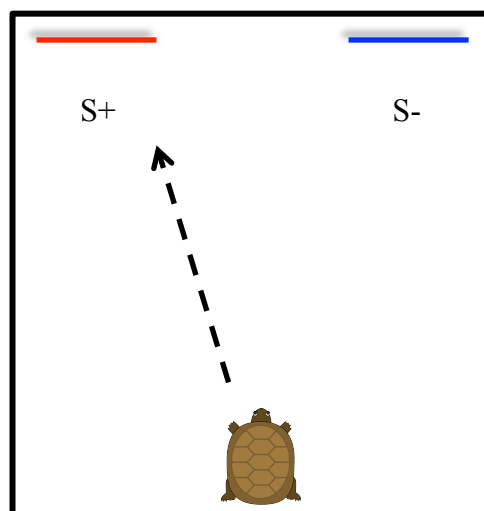
anticipatory activity might be adaptive since most natural food sources are ephemeral (Moore *et al.*, 2011). Extinction time in honey bees, for example, was observed to vary according to the experience of the individual, such that inexperienced bees stopped returning to the non productive food sources after less than 3 days, while the more experienced ones continued visiting the flower at the same time of the day until 5 days after the nectar production was stopped (Moore *et al.*, 2011). The second aim of the present experiment was therefore to investigate the extinction time of the circadian anticipatory activity in the red-footed tortoise. I expected that, in case of quick extinction, in the week after the food delivery time was changed (see method), tortoises' activity would return to that seen in the beginning of the testing.

#### **4.1.2 Method**

Eleven captive adult tortoises were used in the experiment with carapace lengths between 16.7 and 24 cm (mean= 20.14 cm). Tortoises were housed together in a single enclosure measuring 4m x 3m and containing water *ad libitum*, shelters and hot spots with heat and UV lamp; the floor was covered with soil and bark and was dampened once a day. The temperature of the room was kept between 27 and 30 °C, with humidity between the 70 and 80%. Tortoises were fed once a day during the experiment (see below). A week prior the beginning of the experiment, the lights of the room were synchronized with the UV lights in the enclosure and set up to a 12 h cycle, with lights on from 7am to 7pm.

#### 4.1.2.1 Training

Prior the beginning of the experiment, tortoises were trained to associate a specific colour cue to a food reward. During the training phase red-footed tortoises were trained in an experimental arena measuring 1m x 1m, with the floor entirely covered by bark. The arena was located in the same room where the tortoises were housed. Each subject was habituated to the arena prior the start of the experiment with the same procedure as in chapter 2, section 2.3. Eleven different colours were used, so that each tortoise had one rewarding colour (S+) and ten non-rewarding colours (S-) (table 8). Subjects were pre-trained in a two choice task in which they had to choose between the respective S+ and one of the randomly selected S- cues. Both cues were located in the experimental arena at the same distance from the starting point (~1 m) one left and one right with side chosen at random (fig 4.1).



**Fig. 4.1 Example of training set up. In this instance the tortoise had to approach the S+ cue positioned in the left side of the arena in order to get a reward.**

The tortoise had one minute to approach the S+ stimulus from when it was introduced in the arena. The choice was considered made when the tortoise was within 10 cm distance in front of the stimulus and looking toward it. No food was in the arena during the trial to prevent tortoises using olfactory cues. In case of a correct choice, the tortoise was rewarded immediately with a piece of their preferred food (strawberry, mango or dandelion) dropped in front of the cue. When an incorrect choice was made, the tortoise was removed from the arena and held in a cardboard box (30 x 50cm) until the beginning of the subsequent trial (between 30 to 60 seconds after). This was equivalent to a “time out” used in operant studies (Thompson and Moerschbaecher, 1978). The tortoises were given one session of 15 trials per day. The training continued until each subject reached the learning criterion of 13 correct trials over the last 15 trials (80%) in three consecutive sessions (Lopez, 2001). Once all the subjects had reached the criterion the experiment started.

#### *4.1.2.2 Test 1, do tortoises posses anticipatory skills?*

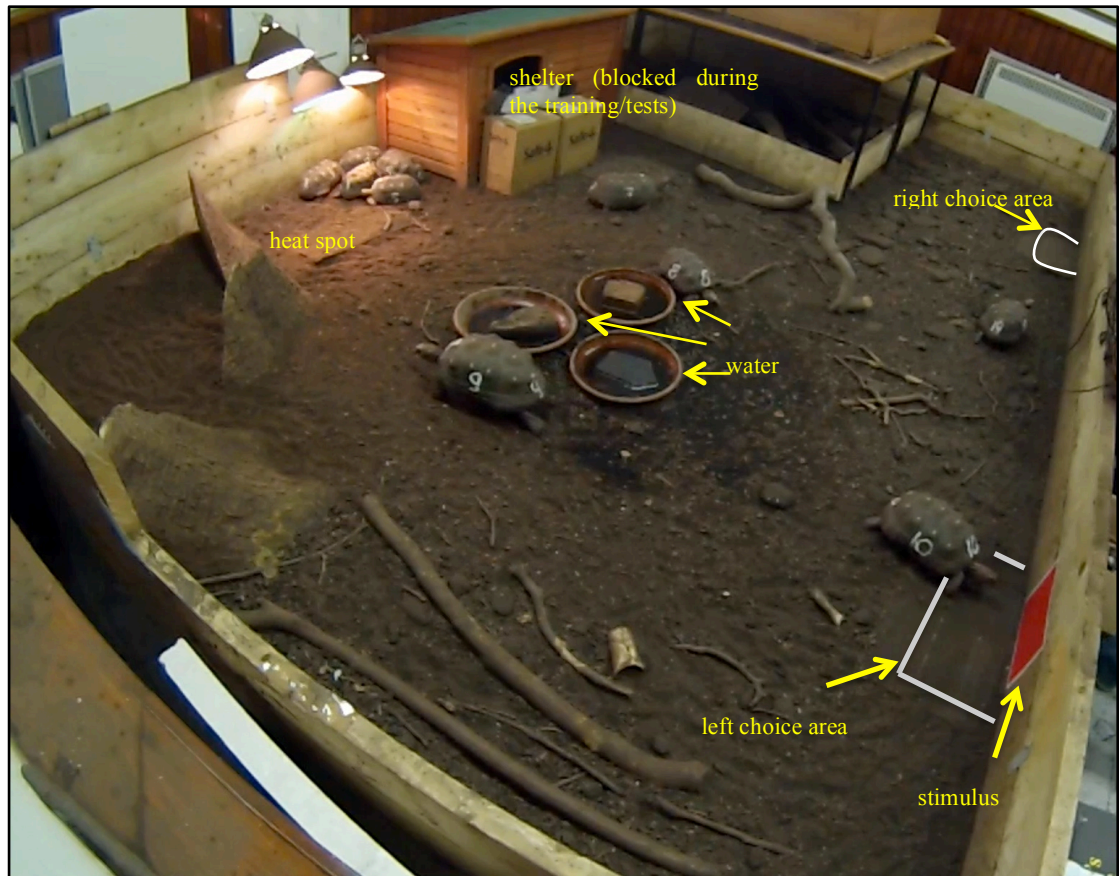
The aim of the experiment was to test whether tortoises express anticipatory behaviours in respect to temporally fixed food delivery events. The food was delivered at intervals of 24 hours (table 8). The training took place in the same enclosure where the tortoises were housed, therefore no habituation was needed (fig 4.2). Every day the experimenter followed a regular routine: at 9am the shelter in the enclosure was closed in order to keep the tortoises in a visible space throughout the duration of the experiment, and the video camera was switched on. Then tortoises were given a warm bath, approximately one minute

long, in order to increase their activity. Tortoises were bathed following a specific order, which was the same order followed for the food delivery time during the experiment (table 8). Each tortoise had a different feeding time, one every 15 minutes (table 8). Because tortoises were housed all together in the experimental arena, this avoided the possibility that the subjects might learn the time of food delivery by copying the behaviour of other individuals. At the established time, one of the 11 coloured cues was presented in the arena, in the right or the left side of the enclosure (table 8; fig 4.2).

**Table 8 Time, colour of the cue and subject order scheduled for the 24h cycle**

<b>7am lights on; 9am camera on</b>			
<b>Food delivery Time</b>	<b>Colour</b>	<b>Tortoise</b>	<b>Side of the enclosure</b>
9.45am	Yellow	1. Moses	Right
10.00am	Blue	2. Wilhelmina	Left
10.15am	Green	3. Aldos	Right
10.30am	Purple	4. Betty	Left
10.45am	Tourquoise	5. Alexandra	Right
11.00am	Orange	6. Savina	Left
11.15am	Pink	7. Patty	Right
11.30am	Brown	8. Darwin	Left
11.45am	White	9. Mozart	Right
12.00am	Red	10. Seisou	Left
12.15am	Black	11. T19	Right
<b>4.30pm camera off; 7pm lights off</b>			

The tortoise pre-trained to approach that colour cue had two minutes to reach the stimulus. In order to define the choice of tortoises, the cue was located over a black rubber mat (50 x 70 cm), where a white line marked at 10 cm around the cue defined the "choice area" (fig 4.2). When the tortoise entered the choice area with its head (fig 4.2), it was removed from the enclosure and fed in an external cardboard box. This was to avoid the possibility that the vision or smell of food could attract the other subjects at the wrong time.



**Fig 4.2 Experimental arena.** The figure shows tortoise number 10, which is responding correctly to its visual cue presented on the left side of the arena.

Tortoises received their daily regular amount of food, ~150g of mixed fresh fruit and vegetable. The cue remained in the arena for a total of three minutes and then was removed. If the tortoise did not pay attention to the stimulus (e.g. it was sleeping or it was oriented in a completely different direction) after two minutes from the established food delivery time, the individual was carried to a distance of 1m from the stimulus (the same distance at which the tortoises were trained to approach the stimulus during the pre-training phase). At this point the tortoise had one minute (as in the pre-training) to go toward the cue and reach the choice area. If this happened, the tortoise was removed from the enclosure and fed as



described above. If it still did not approach the stimulus, it did not receive food for the day and it was left in the arena. In case the tortoise did not approach the stimulus for three consecutive days, on the third day it was removed from the enclosure at the appropriate time, the cue was transferred in the external box and the tortoise was fed in proximity of the stimulus. This assured that all the individuals maintained a normal bodyweight whilst remaining motivated. The tortoise remained in the feeding box until food delivery time of the next individual, i.e. 15 minutes after. When the next stimulus was introduced in the enclosure, the exact same procedure as described above was repeated for the respective tortoise. Once the subject was removed from the enclosure to be fed, the previous tortoise was reintroduced in the arena. This was repeated for every tortoise every morning. The 24 hours feeding cycle continued until 65 consecutive days of test had been completed. The test was constantly recorded from 9am until 4.30pm using a wide-angled camera (GoPro, HERO3<sup>+</sup>, quality of the video 720p60 fps) installed on the wall at the left side of the enclosure. Following test 1 tortoises were given three control tests in order to assess whether they learned to anticipate food delivery time or whether their behaviour was a response to other cues, such as the visual cue introduced in the arena or the movements of the experimenter in the room.

#### *4.1.2.3 Control Test 1, cue conflict test*

This control test aimed to determine whether tortoises actually learned the food delivery time or if they were responding exclusively to the visual cue. To assess this issue the visual stimuli were presented in a different, random, order

than used during the test 1, so that the usual combination between time and colour was uncoupled. The general procedure was the same as described for “test 1” except that the experimenter did not interfere in instances where the tortoises did or did not respond to the colour cue or time. Thus, tortoises were not removed from the arena and did not receive any reward. The control test 1 was repeated three times separated by three days of training (during which the procedure was exactly as in test 1) in-between each control test day (fig 4.3). If the tortoises did not rely on the visual stimuli, I would not expect any difference in the activity pattern between the control test and training days.

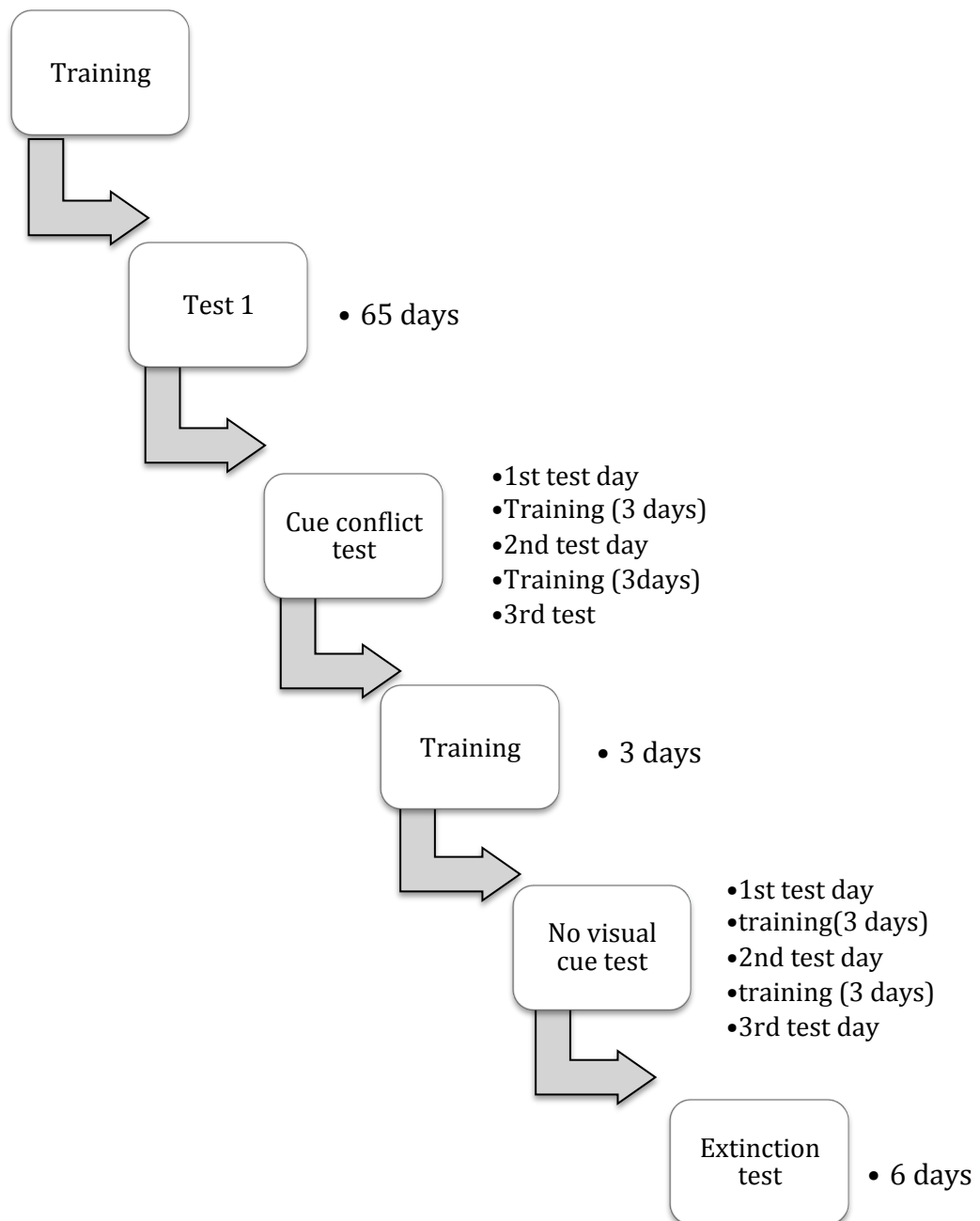
#### *4.1.2.4 Control Test 2, no visual cue test*

This control test aimed to determine the impact of colour cue and the events related to the introduction of it in the arena (e.g. the movement of the experimenter) on tortoises anticipatory behaviour. During this control test tortoises received the initial bath, then no visual cue was presented and the experimenter did not go close to the arena at any time. Tortoises were not removed from the arena at the corresponding food delivery time and did not receive any reward. The control test was repeated three times separated by three days of training (as above) in-between each control test day (fig 4.3). The prediction was that if tortoises rely exclusively on temporal cue to anticipate the food delivery time, I would not expect any difference in the activity pattern between the test and training days.

#### *4.1.2.5 Control Test 3, extinction*

This control test aimed to determine whether tortoises extinguished their anticipatory activity rapidly after a change in the food delivery time. After the completion of the second test, the behaviour of the tortoises was recorded for the subsequent six days. During this period the lights and the camera were switched on and off at the same time as during the previous parts of the experiment. However, the food delivery cycle and regular procedure were stopped: tortoises did not receive the bath at 9.00 am, the visual stimuli were not presented in the arena and tortoises were fed all together after 1.30 pm (fig 4.3). If tortoises extinguished their anticipatory behaviour, I expected a change in their activity in the week after the food delivery time was modified.

**Fig. 4.3 Experimental scheme**



#### 4.1.2.6 Data analysis

##### 4.1.2.6.1 Anticipatory activity

In order to investigate whether tortoises learned to anticipate their food delivery time, the activity of the tortoises during the first and last three days of test 1 was compared: the videos of three days of the first week of test 1 and three days in the last week of test 1 were analysed. The activity (walking or still) of each tortoise was registered for every minute of each video. This means that the videos were stopped every minute and it was registered whether each tortoise was walking or still in this precise instant. Then, the frequency (total number of time each tortoise was active) of the activity of the tortoises during the experiment was used for the analysis. The anticipatory response is commonly measured as an increase of animals' activity in the hour prior the food delivery time (see introduction, section 1.5.5). For the analysis, the frequency of the activity of tortoises during the 74 minutes prior to the food delivery time was used. This time period was chosen because tortoises had different food delivery time during the morning and 74 minutes resulted to be the longest time period at which it was possible to normalise the data.

For the statistical analysis the data were divided in five groups (bins) representing a period of 15 minutes each, so that bin number 1 contained the data regarding the activity (frequency) of all the tortoises during the 15 minutes before their food delivery time and so on, until bin number 5 that contained data from 74 to 60 minutes before tortoises' food delivery time. A generalised linear model, function *glmer* in *lme4* package (Bates, 2010) of R (R Core Team, 2013) with a binomial error, was used to look whether the activity of tortoises was affected by

the interaction between the training period (first and last days of test 1) and the part of the day (the five bins), which were entered into the model as fixed effects. The day and subjects were entered as random terms. P-values were then obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question (Crawley, 2007) and adjusted using Bonferroni correction.

#### 4.1.2.6.2 Control Test 1, cue conflict test

The videos of the three control test days and the three training days preceding each test day were analysed and data regarding the activity of the tortoises (frequency) evaluated in the same way as described in the previous paragraph, i.e. looking at whether the tortoises' activity was affected by the interaction between training / control test periods and the time before the food delivery (five bins). The cue conflict test was designed to test whether the tortoises were actually able to anticipate the food delivery time or if they were responding exclusively to the visual cue exposed in the arena at the food delivery time. If the tortoises did not rely on the visual stimuli, it was expected a non-significant interaction. For the analysis, the training days preceding each control test day were used instead of the last days of test 1, because the control test might have affected the behaviour of tortoises. As further control, the last days of test 1 (before the beginning of the control test) and the training days preceding the control test days were analysed to determine whether the cue conflict test did not have any kind of impact on the behaviour of the tortoises. This was performed in the same way as described the above section (4.1.2.6.1).

Moreover, it was also analysed the number of times each tortoise responded to the correct cue and the latencies of their response, i.e. interval of time from the introduction of the cue in the arena to the moment in which the respective tortoise started to walk towards it, during the training and test days. If tortoises anticipate the food delivery time using exclusively temporal cues, the prediction was that they would not respond to the colour cue when it was introduced at the “wrong” time. If tortoises exclusively used the visual cue as a signal for food delivery, then it would be expected that they would respond to the colour cue no matter the time that it was introduced in the arena and with the same latency of response as in the training. If tortoises were using both temporal and visual cues to anticipate the food delivery event, it would be predicted that tortoises would be more active around the usual food delivery time and if they respond to the visual cue, the latency of the response would be longer than in the training, because, since the cue was introduced at a different time, tortoises should not expect it. In case the tortoises did not approach the cue, a latency of two minutes, i.e. the amount of time the cue was exposed in the arena, was assigned for the analysis.

#### 4.1.2.6.3 Control Test 2, no visual cues test

During the second control test, no visual cues were presented in the arena. This control test aimed to test whether the absence of cues would affect the activity of the tortoises. Once again it was verified whether tortoises’ activity patterns during the control test days and the days of training preceding each control test day were affected by the interaction between training/ control test

period and the time before the food delivery (bins). If tortoises did not rely on cues and external daily events the interaction would not be significant, thus the activity patterns during the control test and training would be same. For the reason explained above, a comparison between the last days of test 1 and the training after the beginning of the no cue test was also made to test whether during the training, tortoises were behaving as they were before starting the tests. The statistical analysis was made using the same method as described above.

#### 4.1.2.6.4 Extinction, tortoises' activity after the food delivery time was changed

As mentioned in the methods, the recordings of the activities of tortoises continued for six days after the end of the “no visual cue” test. In this period food was provided after 1.30pm, tortoises did not receive their morning bath at 9.00am and the visual cues were not presented. The videos were analysed to investigate the activity pattern of tortoises once the food was not on a scheduled time anymore. The information was extracted from the videos in the same way as for the videos of training and tests. For the analysis, the data were divided in two periods of three days in order to maintain consistency with the analysis performed for the previous training and tests, so that the first three days after the change in food delivery time constitute the “period 1” and the following three days the “period 2”. Then, the interaction between (period 1) / (period 2) and the time of the day (bins) were analysed, using a generalised linear mixed model as described above. If tortoises extinguished their anticipatory behaviour quickly, three days or less, the interaction would be significant, indicating a difference in the activity patterns between period1 and period2. The opposite results could indicate that



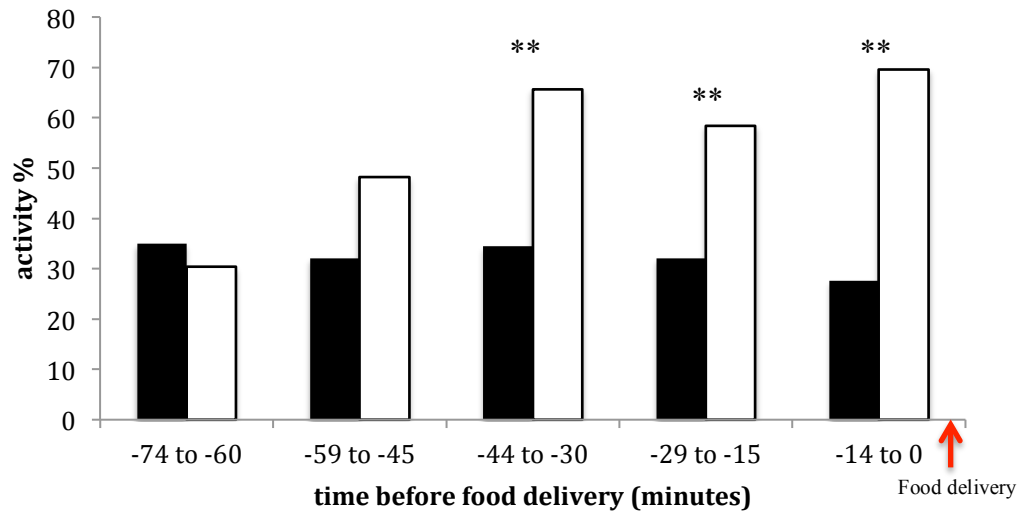
tortoises are not able of extinguish a learned behaviour, or, more likely, that they need more than three days to modify their behaviour after an environmental change.

#### **4.1.3 Results**

Three subjects were excluded from the data analysis because, although they successfully reached the learning criterion during the initial training phase, they never completed the task (i.e. they never reached the stimulus) during the test. Thus, data of a total from eight subjects were analysed.

##### *4.1.3.1 Anticipatory activity*

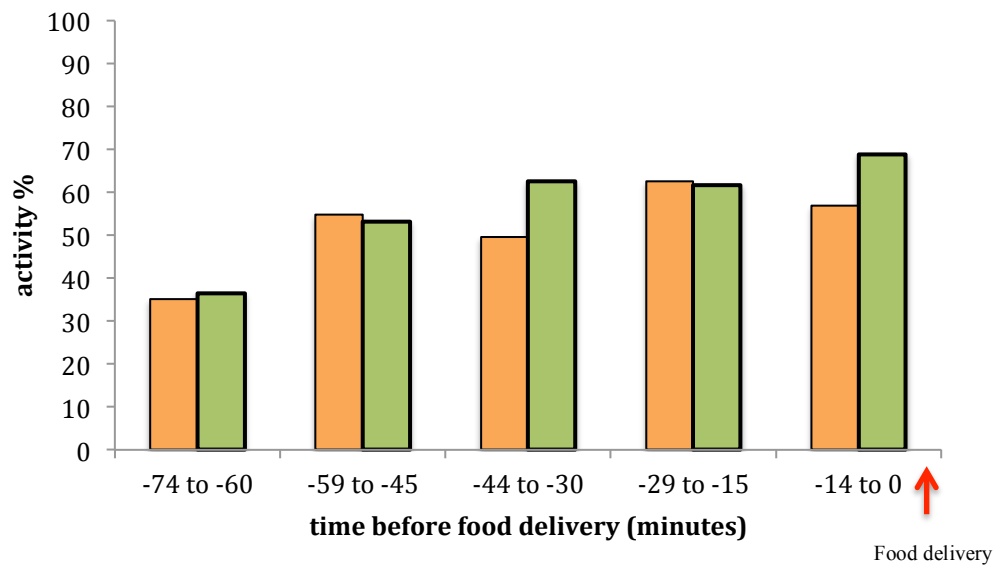
As predicted, an interaction between the period of test 1 (first three days and last three days) and the time to food delivery was found ( $\chi^2(1) = 43.70$ ,  $p < 0.001$ ) (fig 4.4). The activity of the tortoises was found to be higher from 44 minutes before the food delivery time in the last test days than in the first ones (-44 to -30:  $\chi^2(1) = 19.36$ ,  $p < 0.001$ ; -39 to -15:  $\chi^2(1) = 16.62$ ,  $p < 0.001$ , -14 to 0:  $\chi^2(1) = 26.32$ ,  $p < 0.001$ ). Moreover, it was observed that the activity of tortoises did not change throughout the morning in the first test days ( $\chi^2(1) = 1.13$ ,  $p = 0.24$ ) while it did in the last ones ( $\chi^2(1) = 72.83$ ,  $p < 0.001$ ) increasing towards the food delivery time (fig 4.4).



**Figure 4.4** Tortoises percentage of activity in the first three days of test 1 (black) and the last three days of test 1 (white). P-values are reported as Bonferroni adjusted values. Asterisks indicate significance differences between the bins \*\*  $p < 0.01$ .

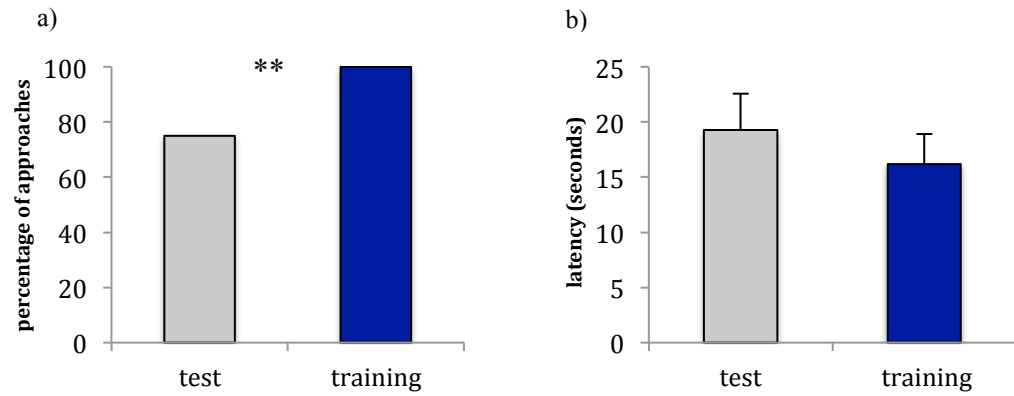
#### 4.1.3.2 Control Test 1: cue conflict test

The activity that the tortoises showed during the training preceding the control test days was not significantly different from the activity showed during the last test 1 days before the control test was started (interaction:  $\chi^2(1) = 2.66$ ,  $p = 0.1027$ ). Thus, we use the data of the training preceding the control test days to compare the activity of tortoises during the control test. An interaction was found between the periods (control test and training) and time to food delivery ( $\chi^2(1) = 4.53$ ,  $p = 0.03$ ). This is probably due the fact that tortoises were slightly less active 44 and 14 minutes before food delivery during the control test. However, no significant difference in activity was found between control test and training time before food delivery time (bins) (fig 4.5). The activity of tortoises followed the same patterns as during training being higher from 59 minutes before food delivery time than 74 minutes before (Fig 4.5).



**Figure 4.5** Tortoises percentage of activity in the cue conflict test (orange) and training (green).

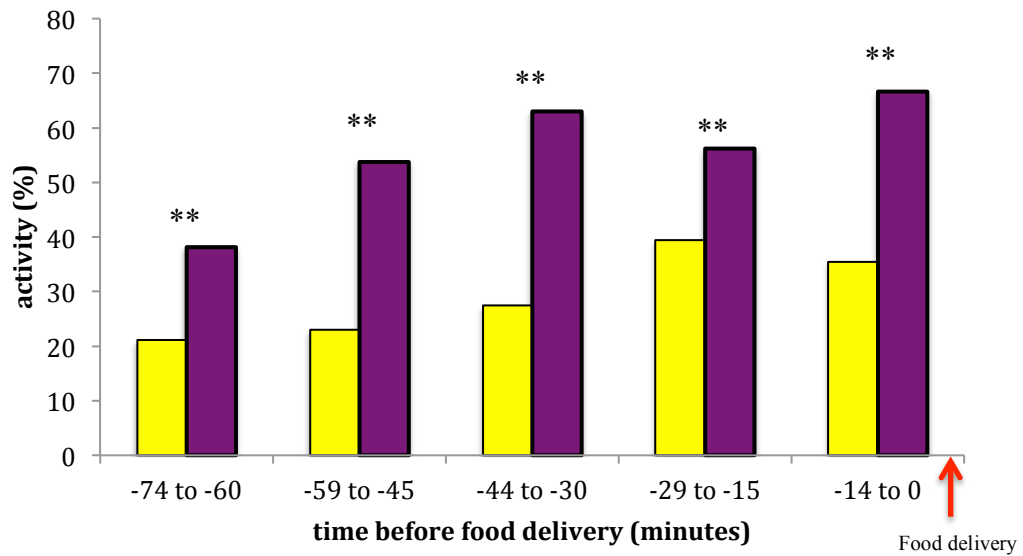
During the cue conflict test, in which the visual stimuli were presented at a different time than usual, tortoises visited their own visual cue significantly less than during the training trials ( $\chi^2(1)=6.67$ ,  $p= 0.009$ ) (fig 4.6a). However, once the tortoises realised that the visual cue was in the arena, the latency of response to the correct colour was the same as in the training ( $\chi^2(1)= 0.7854$ ,  $p= 0.38$ ) (fig 4.6b).



**Figure 4.6.** a) Percentage of times tortoises approaches the correct cue during the control test (grey) and the training (blue). b) Mean  $\pm$  SE latency (in seconds) of response to the visual cue during the control test (grey) and the test (blue). Asterisks indicate significance difference between the groups \*\* $p < 0.01$

#### 4.1.3.3 Control Test 2: no visual cue

An interaction was found between the activity of tortoises during the training preceding the no visual cue test days and the last days of test 1 before the control test was started ( $\chi^2(1) = 5.1995$ ,  $p = 0.02$ ). This might be due to the fact that tortoises were slightly less active in the 39 minutes before the food delivery time during the training preceding the control test days. Therefore, the data of the training before the control test was started were used to compare the activity of tortoises during the test. No interaction was found between the period (test and training) and time to food delivery ( $\chi^2(1) = 2.1461$ ,  $p = 0.1429$ ), suggesting that the trend of the activity of the tortoises followed the same pattern as in the training, increasing toward the food delivery time. However, the tortoises' activity was significantly lower throughout all the periods of time (bins) before food delivery during the test than during the training (-74 to -60:  $\chi^2(1) = 9.63$ ,  $p < 0.001$ ; -59 to -45:  $\chi^2(1) = 32.45$ ,  $p < 0.001$ ; -44 to -30:  $\chi^2(1) = 19.05$ ,  $p < 0.001$ ; -39 to -15:  $\chi^2(1) = 28.31$ ,  $p < 0.001$ , -14 to 0:  $\chi^2(1) = 42.24$ ,  $p < 0.001$ ) (fig 4.7).



**Figure 4.7.** Tortoises percentage of activity in the no visual cue test (yellow) and the training (purple). P-values are reported as Bonferroni adjusted values. Asterisks indicate significance differences between the bins \*\* $p < 0.001$

#### 4.1.3.4 Extinction: tortoises' activity after that the food delivery time was changed

An interaction between Period1 and Period2 was found ( $\chi^2(1) = 39.31, p < 0.001$ ), suggesting that the activity of tortoises was different during these two periods of time. As shown in figure 4.8, in Period1 the activity of the tortoises varies among the different time of the morning (bins) ( $\chi^2(1) = 47.39, p < 0.001$ ), being higher between 74 and 60 minutes prior the experimental food delivery time, then decreases gradually and increase again in the 14 minutes before the learned food delivery time. In Period2 tortoises activity differ over time as well ( $\chi^2(1) = 10.04, p < 0.01$ ) but it follows a linear horizontal pattern as was observed at the very beginning of the experiment (black bars in Fig 4.4).

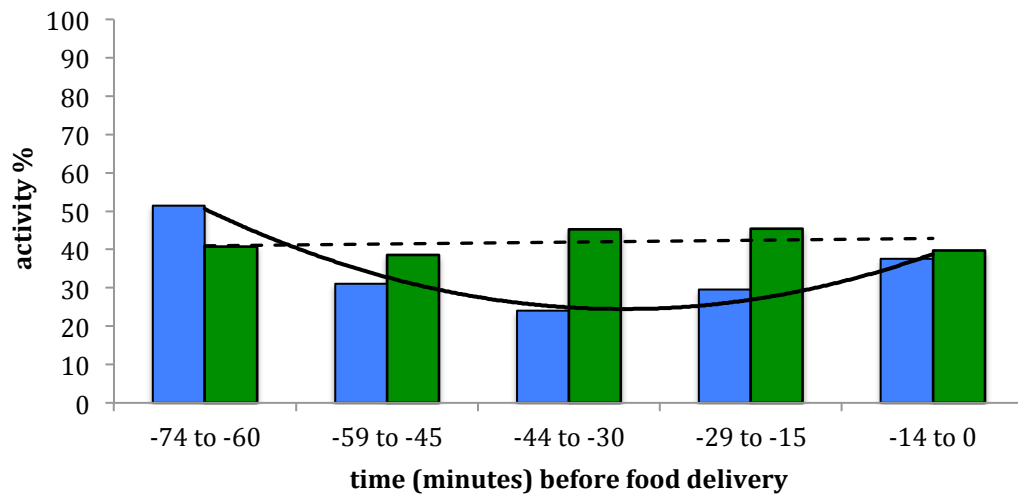


Figure 4.8. Tortoises percentage of activity in the first three days after the food delivery time was changed (period1, blue) and the last three days after the food delivery time was change (period2, green). The lines underline the different trends of the data in the two periods: curve for the data of period1 (solid line), while horizontal for the data of period2 (dotted line).

#### 4.1.4 Discussion

Results showed that tortoises learned to anticipate food delivery time and they expressed it in the same way as mammals increasing their activity in the hour before the food delivery event (e.g. Waite *et al.*, 2001; Roberts, 2002; Smarr *et al.*, 2014). Tortoises were able to adapt their behaviour according to their food delivery schedule, probably using the cues provided by circadian oscillators to indicate when the food was delivered (Roberts, 2002). This was evident from the difference in the activity patterns between the beginning and the end of the test 1: when the experiment first started, tortoises' activity was the same throughout all the morning; after two months (65 trials) the activity patterns changed, increasing towards the food delivery time.

The “cue conflict test” confirmed that the behaviour of tortoises was not solely the result of a response to the visual cues that were introduced in the arena

at the feeding time: when the order of the cues was swapped, tortoises showed the same activity pattern as during training, suggesting that they used temporal cue. However, if they saw the stimulus in the arena, they tended to respond to it with the same latency as during the training. What has to be underlined is that during the training, tortoises never missed their visual cue as they learned at what time to expect it. During the cue conflict test, tortoises visited their stimulus significantly less than in the training, presumably due to the fact that they were not looking for it because they did not expect it at a different time of the day. These results suggest that tortoises learned to use both temporal (circadian oscillators) and visual cues and combined the information of these together to achieve better accuracy in identifying food delivery time. The use of more than one type of cue could be adaptive, because in case of sudden changes, the animal can rely on more than one stimulus to reach a goal (Patterson and Rose 1992; Candolin, 2002, Spottiswoode and Steven, 2010). Thus this suggests that tortoises are potentially able to cope with environmental changes. During the “no visual cue” test, in which the coloured cues and the cues provided by the experimenter were removed, tortoises showed an overall decrease in their activity, but, again, the activity pattern throughout the morning was the same as in the training, increasing towards the time of food delivery. This suggests that tortoises’ activity was affected by the events that happened around them, such as the experimenter’s movements or the colour cues, but their absence did not disrupt their time schedule.

As mentioned in the introduction (section 4.1) plants produce fruit for limited periods (Moskovits and Bjørndal, 1990), thus is very important that frugivores switch rapidly to other productive plants once the one they were

foraging from is not productive anymore (Moore *et al.*, 2011). Data collected after the end of the experiment, when the food was no longer delivered at the established time, suggested that tortoises extinguished their food anticipatory activity after a period of three days. This is in line to what has been found in honey bees (Moore *et al.*, 2011), supporting the hypothesis that a quick extinction of this ability might be adaptive (Moore *et al.*, 2011). Results showed a peak in the activity of tortoises 74 minutes before the food delivery time in period1; this might be due to the fact that tortoises were expecting the morning bath that they were used to receive during the training. This behaviour also disappeared in period2, confirming the fact that tortoises had extinguished what they had learned during the training (Bouton, 1994).

In summary, red-footed tortoises were able to anticipate an event, demonstrating that they are potentially able to learn about time intervals and to return to a food source every 24 hours. However, from the present experiment it is not clear what specific cue or combination of cues triggered their timing mechanism, that means what they used as a landmark to start “counting” the passage of time (e.g the switch on of the lights, the morning bath, the food delivery). Further research is, therefore, necessary to understand more details about how this species perceives the passage of time, looking for example at shorter or longer intervals than the 24 hours cycle or altering the light cycle, and how it might use this skill at its own advantage in its natural environment.



#### 4.1.5 Implications for seed dispersal

The results of this experiment have important implications for seed dispersal. The data suggest that a frugivorous species is potentially able to anticipate the availability of food that follows a fixed diurnal cycle. This would bring advantage to the animals themselves, that would enhance their foraging efficiency, as they would know when to have the best chance to find food at a given time (Henderson *et al.*, 2006). Knowing this, the animal might control its energy expenditure during the day, being more active at the time of food delivery and resting in the other part of the day. Thus being able to anticipate an event would enable energetically optimal searching for food (Moore *et al.*, 2011). Anticipatory skills would lead to an increase in the seed removal rate of the plants, and a consequent decrease of the chances that seed predators would eat their fruit.

The cue conflict test and the no cue test in the experiment gave us important information regarding the possibility that tortoises might combine together information deriving from different environmental stimuli other than the ones given by internal circadian oscillators to perceive the passage of time. In particular, during the no cue test, the overall activity of tortoises dropped. This suggests that the tortoises might have learned about events that were happening in the environment, such as the movement of the experimenter. This is important because it suggests that tortoises might be able to learn about a range of salient environmental cues, such as the presence of flower or the raise in temperature, as indication of imminent presence of food. A study of Janmaat *et al.* (2006), suggests that gray cheeked mangabeys (*Lophocebus albigena johnstonii*) tended

to revisit fruiting trees more frequently after warm periods than after cold ones: high temperatures increase the ripeness of fruits, thus the authors suggested that mangabays use this variable to decide when and where to forage. Honey bees also showed to present food anticipatory activity according to the weather conditions (Moore *et al.*, 2011). If animals are able to use weather as a cue to anticipate presence of food, in this case ripe fruits or nectar, it means that they would be able to reach the food source first defeating competitors, or get there at same time, in case competitors possess the same cognitive skill, increasing their chances to actually find food. Plants in turn, would have guaranteed that fruits would be exploited by frugivores at the right time preventing fruits from falling and laying under the parental tree where the mortality rate is generally higher (Janzen, 1970). Therefore, anticipatory skills would improve seed removal and increase the quantity of seeds dispersed by legitimate seed dispersers, enhancing the reproductive success of trees.

## **4.2 Experiment 7. Creating predictions: how perceiving cycles impacts upon seed dispersal.**

### **4.2.1 Introduction**

In experiment 6 tortoises were shown to be able to anticipate events on a 24 h cycle, and the advantages that this skill might bring to frugivores and plants were discussed. In particular, it was suggested that the ability to anticipate the presence of food in a particular area would enhance the fruit removal rate by dispersers, preventing seeds from remaining under the parental plants or being eaten by seed predators. Thus, anticipatory behaviours in animals may bring benefits to the seed dispersal process that can potentially last during the entire fruiting period of the plant, as animals tend to extinguish their anticipatory activity only when the food is not available anymore (Saunders, 2002; Tautz, 2008; Moore *et al.*, 2011).

However, as mentioned in the introduction of this chapter (section 4.1), plant fruiting period/s are not continuous but follow a regular cycle (Moskovitz and Bjørndal, 1990). Therefore, a step forward would be to know if frugivores are able to learn and remember about cycles longer than the 24 hours cycle tested in experiment 6, because this might allow animals to anticipate seasonal fruiting periods of different plants. This ability might be adaptive because it would allow frugivores to know where to find food in the environment according to the period of the year, providing, in turn, an efficient seed removal service to the plant.

However, to my knowledge, few studies have investigated anticipation of events for periods longer than 24 hours (Gallistel, 1990).

An example that demonstrates how this anticipatory skill could be beneficial for seed dispersal, is the synchronised cycles of migratory birds and plants fruiting periods in the Mediterranean region (Peñuelas *et al.*, 2002). In this area numerous species of plants and migratory birds have synchronised their life cycles, so that birds gained the advantage of finding abundant food supplies once they arrive in the region, while plants gained an abundant and fast seed removal. However, the global warming of the last 25 years had appeared to cause plants to produce fruit nine days earlier than usual and a delay of the arrival of the migratory birds of 15 days. As a consequence, there has been a decoupling of species interactions corresponding to a decrease on both birds and plants' fitness. The birds in fact did not find enough food once they had arrived at the destination, while plants suffered a decrease in seed dispersal (Peñuelas *et al.*, 2002). Considering the small percentage of seeds that survive after dispersal (Schupp, 1988; Andersen, 1999; Crawley, 2000), a small change in the amount of seeds dispersed could have a major impact on plants' fitness.

This example demonstrates the major impact that frugivores can have on seed dispersal if they are able to anticipate the fruiting period of plants, as they would potentially bring the same advantages brought by the synchronization of plants and animals cycles described above. Therefore, in this section, it will be presented a seed dispersal model to test this hypothesis, by simulating an environment with periodic food availability and verifying whether animals with a memory as long as, or longer than, the fruiting cycle of trees would actually bring

to plants more seed dispersal benefits than animals that have no memory about plants' fruiting cycle.

In particular the hypothesis tested were:

H<sub>1</sub>: The interaction between animal memory and plants' cycle length predicts the total amount of seeds dispersed in the environment.

H<sub>2</sub>: The interaction between animal memory and plants' cycle length predicts the survivorship of the animals.

The predictions are that (1) animals with memory that lasts an equal or longer amount of time than plants' cycle length would be able to more successfully relocate the plants when they are fruiting compared to animals with memory shorter than the fruiting cycle of plants, thereby dispersing more seeds. However, if the plant fruiting cycle were longer than the memory retention of the animal, the beneficial effect of memory would gradually decrease until having long-term memory would no longer affect the quantity of seeds dispersed in the environment. (2) The same effect was expected for animals' survival, so that animals with memory equal or longer than plants' cycle length would survive longer than animals with shorter memory. At plants' cycle length longer than animals memory I expect that memory would no longer predict animals' survival rate.

#### 4.2.2 Method

The method of the model is the same as described in chapter 2, section 2.2. The only difference is that the plants appear in the environment at different random cycle lengths (from 1 to 400,  $SD \pm 10$  timesteps) in every run of the model. This means that if, for example, the plants have a cycle length equal to 1, the tree would appear in the environment every timestep, while if they have a cycle length of 350, the plant would appear in the environment every 350 ( $\pm 10$ ) timesteps. These cycle lengths were chosen because animals with memory close to 1 (high memory) have a complete memory decay around the amount of 350 timesteps, thus their memory is slightly shorter than the maximum cycle that can be assumed by plants. The memory decay of animals with lower memory follows an exponential logarithmic distribution; so that memory of 0.5 value will be extinguished in about 50 timesteps and so on until animals with memory close to 0 have an instantaneous memory decay.

**Table 9 . List of the parameters used in the model**

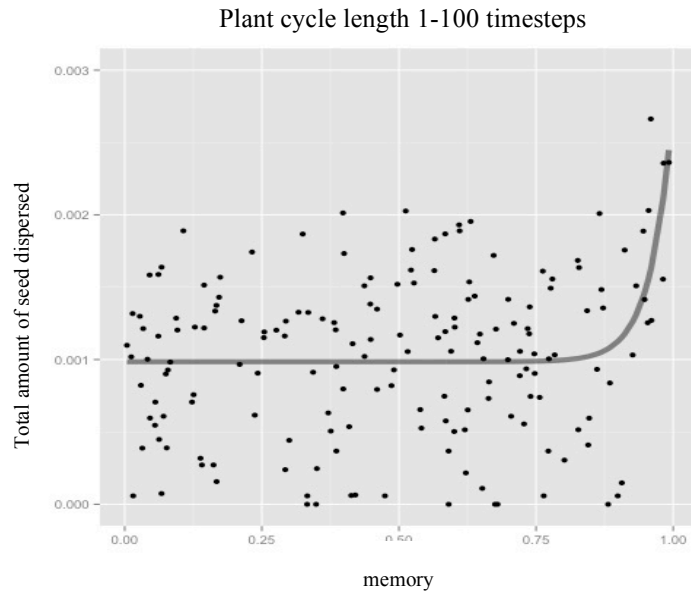
Parameter	Values explored
$\alpha$ : sensory attenuation coefficient	0.5
$\beta$ : memory decay coefficient	Drawn from an exponential logarithm distribution (0.99,1)
$\gamma$ : friction coefficient	0.07
$\kappa$ : satiation coefficient	0.1
$\lambda$ : hunger coefficient	0.99
$\mu_d$ and $\sigma_d$ : parameters of the cumulative lognormal probability density function describing the probability of defecation	4; 0.2
$\mu_s$ and $\sigma_s$ : parameters of the cumulative lognormal probability density function describing the probability of seed excretion	1; 0.2
$T$	10,000

$\kappa_m$ : concentration parameter for von Mises distribution	1.83
<i>Death threshold</i>	0.001
<i>Plant Quantity</i> : value of the plant as it appears in the vegetation map	1
<i>Plant Quality</i> : quality coefficient of the plant	1
<b><i>Plant cycle length</i></b>	<b>1 to 400 timesteps</b>

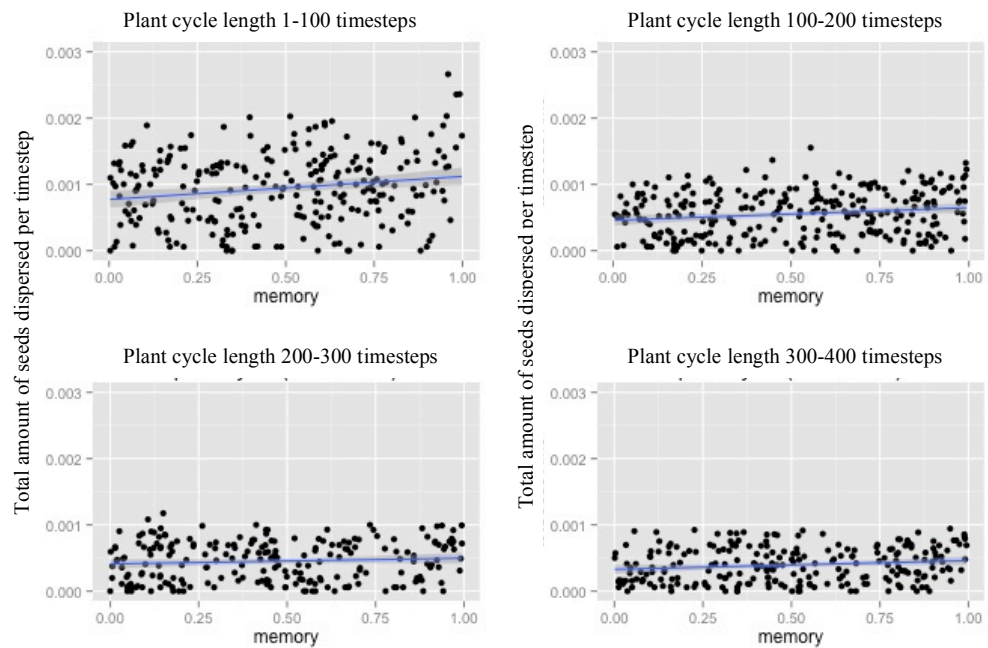
### 4.2.3 Results

#### 4.2.3.1 $H_1$ : Total amount of seed dispersed in the environment

The interaction between animals' memory and the length of the plants' cycle affected the total amount of seeds dispersed in the environment ( $F(1,995)=8.87$ ,  $p<0.01$ ). Figure 4.9 shows that, when the plant cycle is from 1 to 100 timesteps long, the shape of the data follows an exponential curve. The amount of seeds increases exponentially at the higher level of memory. For cycle lengths longer than 100 timesteps, the effect of memory decreases but still animals with more memory provide a better seed dispersal service, until, at a cycle length longer than 300 timesteps, the amount of seeds dispersed becomes the same no matter the level of memory ( $F(1,996)=1.57$ ,  $p<0.21$ ) (Fig 4.10).



**Fig 4.9** Effects of memory on the amount of seeds dispersed per timestep at plant cycle lengths from 1 to 100 timesteps. The dots represent each model run. The line delineates the exponential trend of the data.

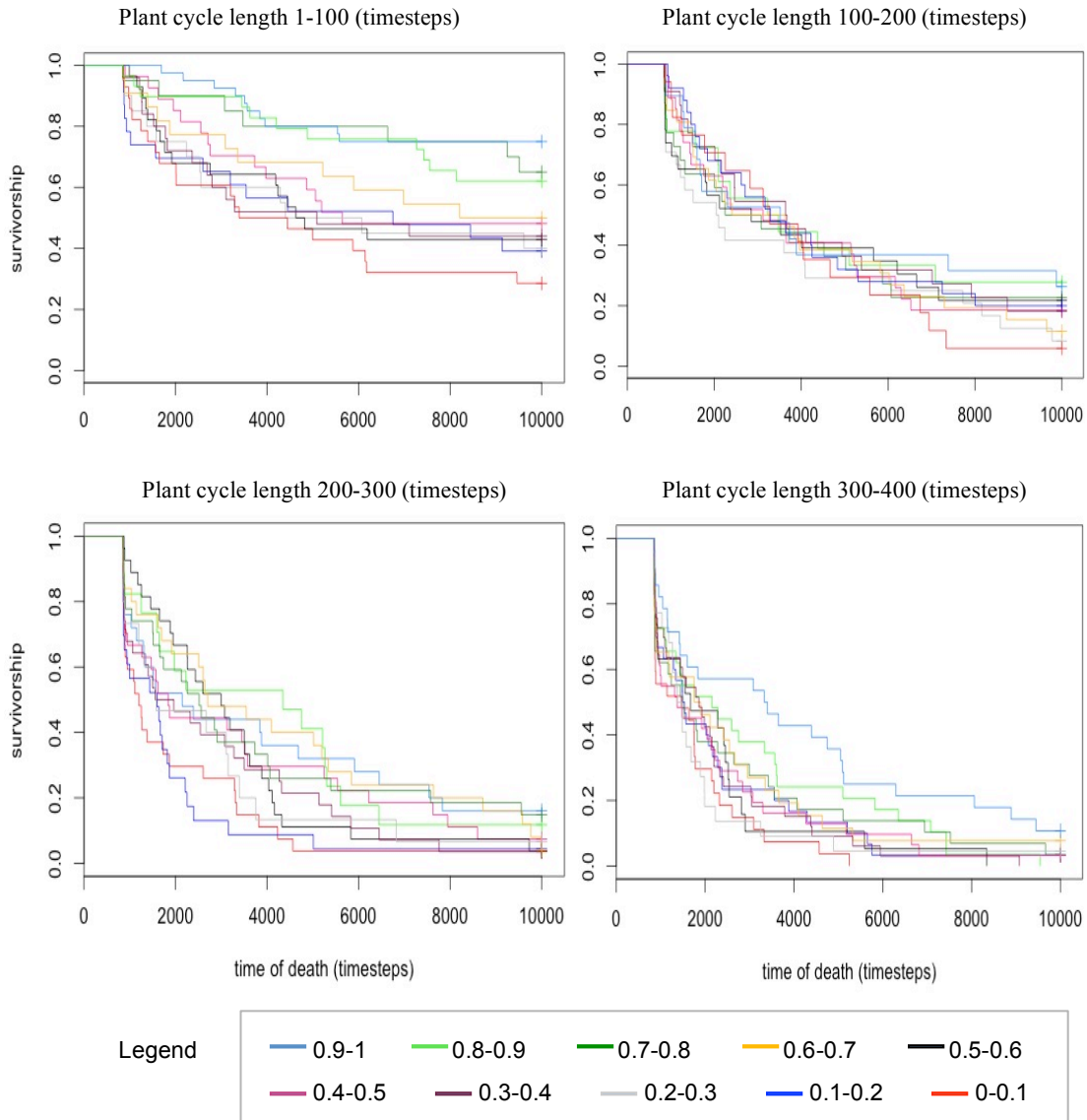


**Figure 4.10** Effects of memory on the amount of seeds dispersed per timestep at different plant cycle lengths. The dots represent each model run. The blue line indicates the average amount of seeds dispersed.



#### 4.2.3.2 $H_2$ : Animals' survival

Animal survival was not predicted by the interaction between memory and plants' cycle length ( $\chi^2(7, N=1000)=307.8, p= 0.43$ ). However, memory and plants' cycle length are significant as main effects (respectively:  $\chi^2(3, N=1000)=293.4, p< 0.001$ ;  $\chi^2(3, N=1000)= 276.7, p< 0.001$ ). This means that animals with higher memory survive longer than animals with lower memory. In addition, the survival rate of the animals is higher in environment where plants' cycle is short than in environments where plants have long fruiting cycle (fig 4.11).



**Figure 4.11** Survivorship of animals with different memory in environment with different plants' cycle length. The legend at the bottom indicates the range of the values of memory in which the data were grouped exclusively for a clearer representation (0-0.1= low memory; 0.9-1= high memory).

#### 4.2.4 Discussion

Plants follow fruiting cycles that have different length according to the species (Moskovitz and Bjorndal, 1990; Peñuelas *et al.*, 2002). The model presented here aimed to compare between the different amount of seeds dispersed by animals that can remember about a plant's cycle and those animals that cannot. From the results it is clear that animal that possess longer memory are able to disperse more seeds than animals with lower memory when the plants' cycle length is under 200 timesteps. However, when the plants' cycles is longer than 200 timesteps and so longer than animals' memory, the benefits brought by memory decrease until they disappear entirely, leaving the amount of seed dispersed subject to random events. Thus, these results underline the importance that animals' memory about fruiting cycle might have in enhancing the seed dispersal process, as well the potential vulnerability if animals memory is disrupted. As mentioned previously, a contemporary problem faced by many ecosystems is that plants are suffering changes in their flowering and fruiting cycles as a consequence of changes in temperature and precipitations (Sillet *et al.*, 2000; Peñuelas *et al.*, 2002) causing a decoupling of species interactions, such that plants are losing pollinators and seed dispersers, while foragers are losing their food supplies (Fitter *et al.*, 1995; Peñuelas *et al.*, 2002). Thus, knowing more about animals' ability to learn and anticipate infradian cycle (longer than 24h) could provide some insight to support this issue. The model showed also that animals with longer memory are able to survive longer than animals with shorter memory in every scenario, as was found as well in the models in the previous chapters. However, no interaction between memory and plants' cycle length was found, in fact, despite the memory level, the survival rate drops accordingly to the

plants' cycle length (fig 4.11). This means that, memory has an effect on survival rate also when the plant cycle length is longer than memory itself. This might be due to the fact that they tend to explore the environment less at random than animals with low memory (see chapter 2), thus they have higher chances to be close to the location where the plant will “appear” and find it once it is fruiting.

From the results of the model it could be hypothesised that plants that present shorter fruiting cycles received more dispersal benefits than plants with longer cycles; in fact plants with cycles shorter than 100 timesteps had a larger amount of seeds dispersed even from animals with lower memory. Thus, plants that present fruits more than once in a year would receive a double advantage: firstly, by producing more fruit, the total amount of seed dispersed would be bigger than from producing fruit only once per year. Secondly, plants with shorter cycles would have more probability to be revisited by animals with or without memory, which would again increase the amount of seed dispersed. However, producing fruit is costly (Snow *et al.*, 1989; Ashman, 1994) and also from the frugivores point of view, long-term memory encompasses a cost (Mary and Kawecki, 2005). As a consequence, field and cognitive studies might reveal that retaining information about the location of plants that have long fruiting cycles might not be an advantage, in particular in environments where plants with short fruiting cycle are also present. Probably, returning to the same food source every 24 h until the food is not available and then switch rapidly to a new one, as seen in the previous experiment (section 4.1), might be the most convenient strategy for frugivores.

### 4.3 General discussion

In this chapter it was explained how frugivores' ability of anticipating events and plant fruiting cycles might impact upon seed dispersal. In experiment 6, the ability of the tortoise *C. carbonaria* to anticipate food delivery on a 24 hour cycle was investigated; tortoises developed a food anticipatory activity, as was shown in mammals and birds (Stephan, 1979; Mistlberger, 1993; Mistlberger, 2009; Waite *et al.*, 2001; Pendergast *et al.*, 2009), meaning that they were able to anticipate the food delivery time on a circadian cycle. Then, the hypothesis that anticipating fruiting periods of trees after longer periods of time than 24 hours would lead to an increase in the amount of seed dispersed from plants was tested in a seed dispersal model (experiment 7). Results of the model showed that, as predicted, animals that have memory at least as long as the plant cycle are able to disperse significantly bigger amount of seeds from plants than animals with no memory. Moreover, memory allows animals to survive longer also in environment where plants' fruiting cycles are longer than animals' memory itself, therefore memory, despite its cost (Mary and Kawecki, 2005), may represent an evolutionary advantage.

The results of this chapter underline the importance that perceiving the passage of time in order to anticipate fruiting events on a daily and seasonal scale would have on seed dispersal by substantially increase the amount and rate of seed removal from plants. However, in literature the debate about animal sensitivity of the passage of time, in particular for periods longer than 24h, is still open: although animals possess long-term memory for specific tasks (e.g spatial memory, see chapter 2 and discriminatory skills, see chapter 3) it is difficult to

define whether they possess a concept of time that allows them to use their memories of past events to make long-term future decisions, e.g. where to find food in three months (Roberts, 2002). More information about how animals might achieve long-term anticipatory skills is needed to define with more precision the effects that frugivores might have on plant fitness.

# CHAPTER 5

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## 5. SEED DISPERSAL IN FOREST GAPS

### 5.1 Experiment 8. Do tortoises increase seed dispersal in forest gaps?

#### 5.1.1 Introduction

In the previous chapters the cognitive abilities of tortoises were examined and it was tested how different lengths and types of memory would impact upon seed dispersal using seed dispersal models. In this chapter it will be presented a seed dispersal model parameterised specifically with the data on red-footed tortoises' cognition collected in the previous chapters with the aim to test one of the characteristics that makes tortoises unusual as seed dispersal vectors: the use of gaps in the forest.

Red-footed tortoises, are considered good seed dispersers for multiple reasons: they eat a large variety of fruit (Moskovitz and Bjorndal, 1990; Strong and Fragoso 2006; Wang, 2011), they swallow the seeds minimising the chances of damaging them (Moskovitz and Bjorndal, 1990; Strong and Fragoso 2006), seed viability after the gut passage is very high (between 91% and 100 %, Strong and Fragoso 2006) and, although daily movements are relatively limited

(average= 57m  $\pm$  102m; Strong and Fragoso, 2006), their gut retention time is long (1.6 to 6.6 days, Moskovits 1985; Strong and Fragoso 2006), which results in a respectable dispersal distance (average 174m, Moskovitz and Kiester, 1987; Jerozolinski *et al.*, 2009). In particular, one characteristic makes this tortoise, and other frugivores reptiles, unique as seed dispersers: the active use of gaps in the forest (Greenberg, 2001). Gaps are defined as “canopy openings extending through all levels down to an average height of 2 m above ground” (Brokaw, 1982 in Popma *et al.*, 1988). Thus, gaps are holes in the foliage, created by trees or branch falls, which create spots in the forest where light can directly reach the soil. Reptiles need direct sunlight and warm spots to thermo-regulate; these are provided by forest gaps. There is evidence that the abundant light and availability of bare ground in newly formed gaps positively influenced reptile abundance in forest environments, which is the 3% higher than in closed canopy areas (Mushinsky, 1985; Greenberg, 2001). From many plants’ perspective, gaps represent good deposition site as they offer light and a lower competition for resources than the closed canopy areas. For example, forest gaps favour the establishment of seedlings of shade-intolerant plants and the achievement of the mature stage of shade-tolerant plants, which persist beneath the forest canopy as suppressed juveniles until they receive more light (Shupp *et al.*, 1989). Thus, gaps have an important function for the regeneration of tropical rain forest plants (Denslow 1980; Popma *et al.*, 1988; Karsten *et al.*, 2013). However, the arrival of seeds in gaps, in particular if large in size, is rare (Howe and Miriti, 2004). This happens because gaps do not offer advantages for non-reptilian frugivores, which tend to avoid them, in particular if recently created (Schupp *et al.*, 1989). Newly formed gaps do not offer fruits for frugivores, they do not offer protection from

predators as they are open areas (Snow and Snow, 1986; Schupp *et al.*, 1989) and they do not offer branches that can be used by birds and monkeys as perches (Schupp *et al.*, 1989; Bélisle *et al.*, 2001). As a result, only the edges of the gaps are likely to receive high seed deposition as the animals can find refuge from predators in the nearby canopy (Snow and Snow, 1986; Schupp *et al.*, 1989). The same situation is even more extreme in areas disturbed by humans' activity, such as deforested areas, which suffer very low seed deposition due to rare visits by frugivores (Howe and Miriti, 2004). Deforested areas are usually larger than naturally formed forest gaps, and the lack of endozoochory events there is even more severe. This limits the possibility of reforestation of such areas, and affects the dispersal of primary forest species with large seeds (Turner and Corlett, 1996), which results in a preponderance of small-seeded pioneer species (Wunderle, 1997). One solution to this problem consists in planting perches in the centre of deforested areas in order to attract birds and promote seed dispersal (Miriti, 1998; Holl 1999) with the aim to accelerate the growth of corridors, which, in turns, would attract more frugivores (Howe and Mitiri, 2004). However, wind and reptiles may be the major cause of seed dispersal in gaps and deforested areas (Kimmins, 1987; Schupp *et al.*, 1989). As a consequence, frugivorous reptiles are likely to be important resources, as they are among the few dispersers that directly deposit seeds in gaps helping the forest restoration process. Despite the importance of the issue, to my knowledge, no studies have directly investigated the impact of reptiles upon seed dispersal in tropical forests' gaps.

Therefore, this chapter aims to test the impact of red-footed tortoises on seed dispersal in forest gaps. Two different types of environments were simulated: the first represents a closed canopy forest with occasional gaps; the second simulates



an environment with wide deforested areas. As a control, the same simulation was then repeated with a modelled animal with the same cognitive and ecological characteristics of a red-footed tortoise, but that behave like other non-reptilian frugivores, which means not being attracted by gaps, but visiting them only by chance. I predict that animals that actively use gaps, as red-footed tortoise, would deposit more seeds in the gaps in both types of environments; in particular I expect that the amount of seeds dispersed in gaps is predicted by the interaction between the type of animal and type of landscape.

### **5.1.2 Method**

The structure of the model is exactly the same as described in chapter 2. However, the cognitive abilities of the animal were parameterised using data collected in the previous experiments with red-footed tortoises (table 10). The landscapes were modified as described below.

#### *5.1.2.1 Animals that use gaps*

Animals' movements, perception, memory, feeding, gut passage and seed dispersal functioned in the same way as described in chapter 2. However, in this model memory has a fix value (0.99) instead of covering a range (from 0 to 1). The value of memory chosen is close to one, which means almost perfect memory. This because each model run has 10000 timesteps; assuming that (1) a timestep corresponds to a minute of time, (2) that tortoises move exclusively during the day (12 hours) (Moskovitz and Bjorndal, 1990) and (3) tortoises are

active on average the 50% of the day (Guzman and Stevenson, 2008), 10000 timesteps would be equivalent to one month of tortoise activity. The literature and experiments 1 and 4 (respectively chapters 2 and 3) showed that tortoises retain spatial information for at least two months and visual information for much longer, over a year. For this reason the memory was set as perfect (0.99).

#### *5.1.2.2 Landscape*

Two types of landscape were simulated: closed canopy and deforested environment. Gaussian random landscapes (1000 for the close canopy and 1000 for the deforested environment) of dimension 100 x 100 hexagons, were created using the function `gstat` in R setting the `range= 3` for the close canopy and `range= 6` for the deforested environment (Crawley, 2007), and then uploaded to MATLAB. Every hexagon of the landscape is equivalent to one metre of land, thus the total dimension corresponds to a plot of forest of one hectare. This measure was chosen because it contains the possible landscape travelled by red-footed tortoises in a month (Guzmán and Stevenson, 2008). Dimensions and frequency of gaps in the closed canopy landscape, and the proportion of areas occupied by adult plants (30%), were simulated according to data collected in a neotropical rain forest located between Esmeraldas and Imbabura Provinces in Ecuador (79° 02' 756" West, 0° 20' 867" north) by Ana Mariscal and colleagues (unpublished data). The shapes and sizes of the deforested areas in the deforested landscapes were simulated at random. The hexagons forming the gaps had a value of 1 (maximum attractiveness); the hexagons occupied by plants had a random value between 0 to 1; all the other hexagons had a value of 0 (minimum

attractiveness). The fruiting plants in each landscape were equivalent to the 30% of the canopy and located at random in the landscape, but not inside the gaps (fig 5.2).

In the control environment, with animals visiting the forest gaps by chance, the values of the gaps were changed to 0 (instead of 1 as previously explained). In this way, to the eyes of the animal, gaps had the same attractiveness as the other part of the landscape not covered by plants.

**Table 10 List of the parameters used in the tortoises' model**

Parameter	Values explored
$\alpha$ : sensory attenuation coefficient	0.5
<b><math>\beta</math>: memory decay coefficient</b>	<b>Fixed value= 0.99</b>
$\gamma$ : friction coefficient	0.07
$\kappa$ : satiation coefficient	0.1
$\lambda$ : hunger coefficient	0.99
$\mu_d$ and $\sigma_d$ : parameters of the cumulative lognormal probability density function describing the probability of defecation	4; 0.2
$\mu_s$ and $\sigma_s$ : parameters of the cumulative lognormal probability density function describing the probability of seed excretion	1; 0.2
$T$	10,000
$\kappa_m$ : concentration parameter for von Mises distribution	1.83
<i>Death threshold</i>	0.001
<b>Landscape size</b>	<b>100 x 100</b>
<b><i>Plant Quantity</i>: value of the plant as it appears in the vegetation map</b>	<b>Random value from 0 (min quantity) to 1 (max quantity)</b>

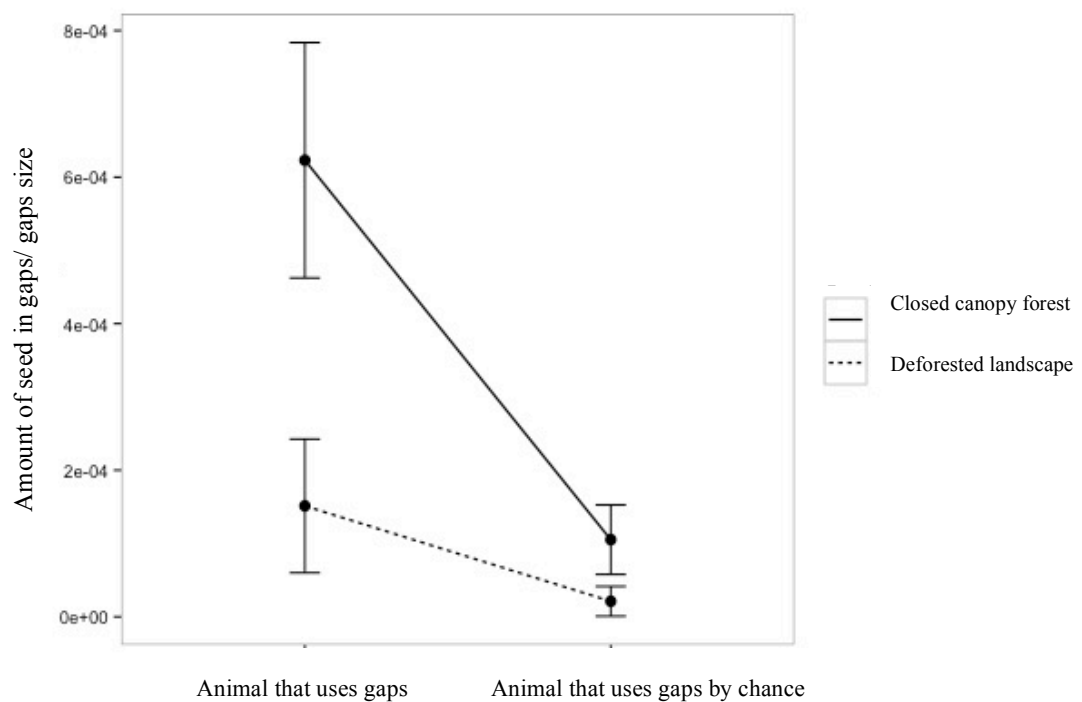
#### 5.1.2.3 Data analysis

Data were analysed by creating a linear model (function `lm` in R, Crawley, 2005) to test whether the amount of seeds dispersed in the gaps was predicted by

the interaction between the animal (gap user or random gap user) and the type of landscape (closed canopy or deforested), which were entered as fix terms. To assess the p-value, this model was compared with an equal model without the interaction with likelihood ratio tests.

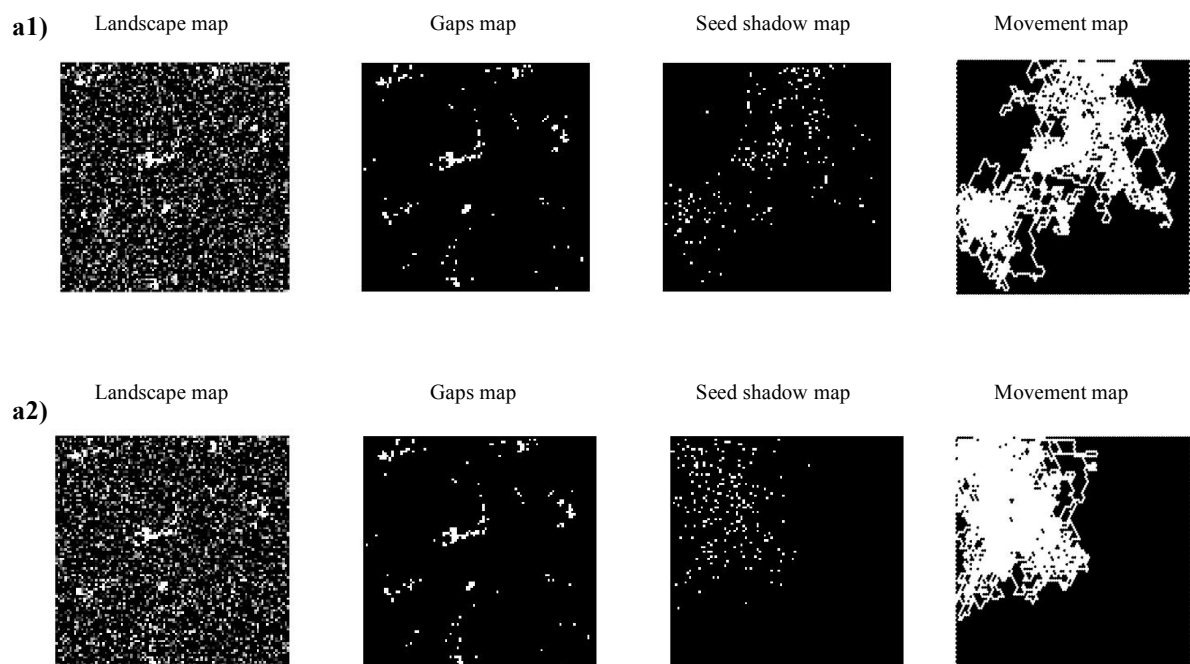
### 5.1.3 Results

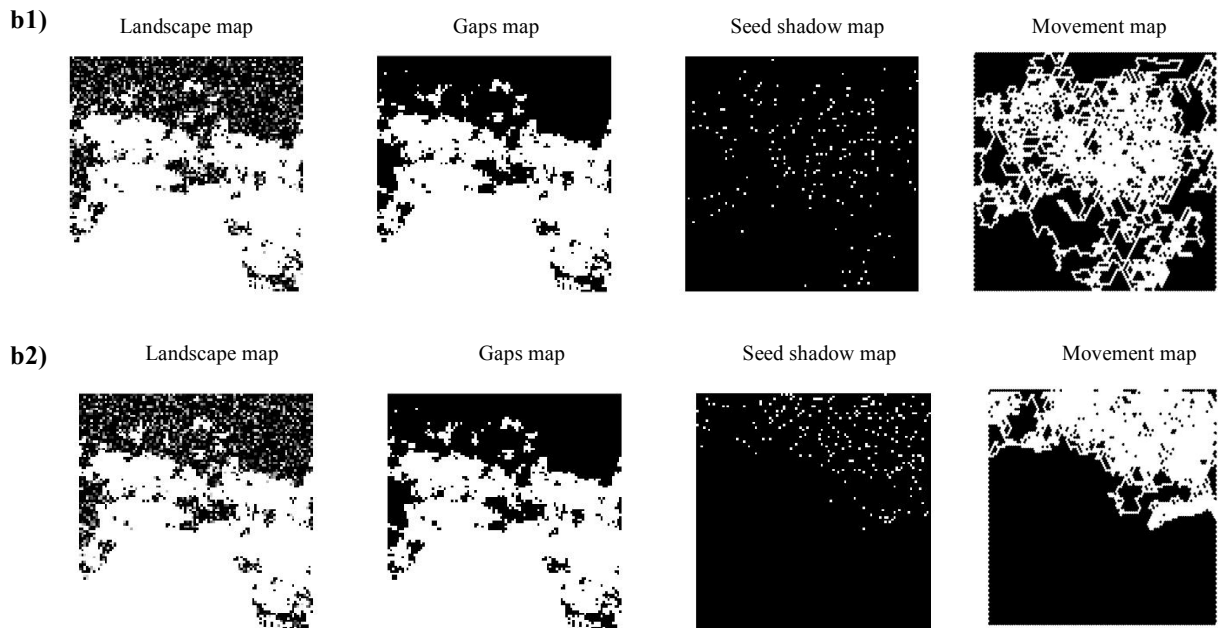
As predicted, the proportion of seeds deposited in the gaps and deforested areas was affected by the interaction between the animal (gap user or random gap user) and type of environment (closed canopy or deforested environment) ( $F(3, 3996) = 6643, p < 0.001$ ) (Fig 5.1), so that the amount of seeds dispersed in the gaps is higher in closed canopy forests inhabited by animals that use gaps.



**Fig 5.1** Amount of seeds dispersed in the gaps relative to the gaps size by animals that actively use gaps and animals that use gaps by chance (control)  $\pm$  SD in two different landscapes.

Figure 5.2 shows an example of the types of landscape (closed canopy (a) and deforested environment (b)) with animals that use gaps (a1,b1) and use gaps by chance (a2, b2). The movement map shows how animals that use gaps travel more inside gaps than the control animals. This is particularly visible in the deforested landscapes, in which it is evident that control animals tend limit their movements to the edges of the deforested areas, while animals that use gaps explore also the centre of them. As a consequence the seed shadow resulting from animals that uses gaps is spread also throughout the deforested areas.





**Fig 5.2** Maps of closed canopy forest (a1, a2) and deforested environment (b1, b2) with seed shadows and movements of animals that use gaps (a1, b1) and that use gaps at random (a2, b2). The white part in the gaps map indicate the gaps; the white dots in the landscape maps indicate the plants; the white dots in the seed shadow maps indicates the seed dispersed; the white part in the movement maps indicate animals' movements.

#### 5.1.4 Discussion

This chapter presented a model parameterised with red-footed tortoise data, with the aim to test the impact that frugivorous reptiles may have on the seed dispersal, in particular in terms of depositing seeds in forest gaps and deforested areas. The results showed that an animals with the cognitive ability and behaviour (use of gaps) of a red-footed tortoise would disperse significantly more seeds in gaps and deforested areas than animals that visit these only by chance (control).

The first thing to be noticed is that, according to the model, both types of animals (gap user and control) disperse more seeds in gaps inside the closed canopy forest than in deforested areas. This probably happens, because plants

surround them, thus the animal can forage frequently and then go back to the gap, or simply comes across the gap while foraging. This does not happen in deforested environments in which the deforested area is concentrated in a particular part of the landscape creating areas totally isolated from vegetation. In these cases, it can be noticed that the control animals tend to randomly visit the edges of deforested areas. As a consequence seeds are dispersed on the edges but there are not dispersal events in the centre of the deforested site. This prediction of the model is in line with what found in Schupp *et al.* (1989) who suggested that frugivores tend to stay at the edges of areas with no vegetation in order to have easy refuge from predators given by the proximity of the canopy. These results could have a major impact on deforestation strategies: it might be suggested that by removing trees in smaller patches with closer edges, instead of a single area with a very wide extension, would help a faster reforestation afterward.

The model also highlights the active role played by animals that use gaps in the restoration of forest: the simulation shows how they travel and deposit seeds even in the middle of deforested areas. This would have a major influence on the probability of seedling establishment in the central areas of gaps, which would lead to the creation of corridors, which would encourage other frugivores to travel in those areas. As a result, the amount of seeds deposited would increase creating a positive feedback on frugivores' arrival. Thus, tortoises could potentially provide the same reforestation advantages brought by human intervention described in the introduction (Schupp *et al.*, 1989; Howe and Mitiri, 2004). This suggests that frugivorous reptiles can potentially make a difference in the reforestation process: the active transportation of seeds into gaps and deforested areas would speed up the closure these sites, with a direct action,

actively depositing seeds, and also by indirect action, as the establishment of juvenile plants (maturing gap) is enough to attract more birds and primates frugivores (Popma *et al.*, 1988; Schupp *et al.*, 1989).

These findings add further evidence to the idea that red-footed tortoises are important dispersers in their habitat (Moskovitz and Bjorndal, 1990; Strong and Fragoso 2006; Wang *et al.*, 2011). Not only do they possess cognitive skills that potentially allow them to discriminate and locate food sources in space time, all of which brings seed dispersal benefits (as described in chapters 2,3 and 4), but, because of their thermo-regulatory needs, they also use gaps which could be key in processes such as forest regeneration.



# CHAPTER 6

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## 6. GENERAL DISCUSSION

### 6.1 Summary of findings, implications and future directions

Seed dispersal by endozoochory is a complicated mechanism. Many studies have analysed the ecological aspects of it (e.g. Schupp, 1993; Link and Di Fiore, 2006; Strong and Fragoso, 2006; Jerozolinski *et al.*, 2009), however, the influence of learning and cognition on animal decision-making have been poorly explored. Animals are likely to use a multitude of cognitive skills for successful foraging, which in turn could affect where they access food and disperse seeds. Thus, in this thesis, I investigated the impact of animal learning and memory on the seed dispersal process.

#### 6.1.1 Where? The impact on spatial learning on seed dispersal

The first question I investigated in chapter 2 was whether the ability of frugivores to relocate food sources in the environment impacts upon the seed shadow of a plant. Knowing whether an animal is able to relocate food sources in space would provide information about which paths animals are likely to take, and can help to predict the distance and quality of seed deposition sites (Wang and Smith, 2002).

Therefore, I investigated the spatial learning and memory of a frugivorous model species, the red-footed tortoise, in a spatial egocentric task (experiment 1). The results add important information to the literature about spatial navigation and memory of chelonians: red footed tortoises was able to relocate a food source in space, and remembered the correct location of food after two months retention time interval. This suggests that the red-footed tortoise is potentially able to return to a food source previously visited for the duration of tropical fruit crop, which is about two-three months (Moskovitz and Bjorndal, 1990; Chapman *et al.*, 2005). However, the subjects failed to solve the same task after 8 months retention time interval. This can have two explanations: (1) as long-term memory is a cost (Mary and Kawecki, 2005), tortoises retain spatial information only for a period that is advantageous, such as the length of a fruit crop (Moskovitz and Bjorndal, 1990; Chapman *et al.*, 2005), as there is no benefit in returning to a food source that has been depleted. (2) Tortoises failed in the task because they did not have any visual cues that might have helped them to remember the location of food after such a long time. Tortoises excel in visual tasks (Burghardt, 1977), and previous studies showed that they can use landmarks to orientate (Lopez *et al.*, 2001; Wilkinson *et al.*, 2009). Thus, it is possible that in environments that provide many visual cues, such as the tropical forest, tortoises would be able to relocate food sources for longer periods than two months.

In order to verify whether frugivores with different spatial memory skills impact differently on seed dispersal I used a seed dispersal model. As expected, results showed that animals with different memory skills provide different seed dispersal services. In particular frugivores with longer spatial memory (slower memory decay) were able to relocate more efficiently the food sources in the

environment than animals with shorter memory (faster memory decay). As a consequence, animals with longer spatial memory: (1) disperse a bigger amount of seeds in the environment; (2) provide a more even distribution of fruit removal from different trees in the landscape; (3) travel less randomly around the environment; (4) provide shorter seed dispersal distance; and (5) survive longer than animals with shorter memory. These results imply that cognition plays an important role in seed dispersal. They show that the ability of an animal to efficiently relocate a plant alters the seed shadow of that plant. This adds information to the previous seed dispersal literature that has only considered the physiological and/or behavioural features of the animal when evaluating their potential as a disperser (Schupp, 1993; Link and Di Fiore, 2006; Strong and Fragoso, 2006; Russo *et al.*, 2006; Jerozolinski *et al.*, 2009). For example, animals of bigger size are usually considered able to bring seed further away from the parental tree than smaller animals because they usually travel greater daily distances (Nathan *et al.*, 2008; Wotton and Kelly, 2012). However, this might not be true because if big size animals tend to often re-visit the same food sources thanks to their spatial memory ability, the dispersal distances at which they will deposit the seeds might be shorter than the ones provided by smaller animals that possess shorter memory and, thus, travel more randomly in the environment. Therefore, results suggest that the incorporation of spatial cognitive skills in the evaluation of plants' seed shadow might bring different results than expected by using the common methods used in endozoochory studies, i.e. combining together the daily distance travelled by the animals and the gut retention time (e.g. Schupp, 1993; Link and Di Fiore, 2006; Strong and Fragoso, 2006).

Therefore, more information about frugivores' spatial memory skills are essential, because there is the evidence that animals with different spatial memory skills bring different kind of services to the seed dispersal processes, and knowing for how long an animal is able to remember the location of a food source, would give us the possibility to predict the impact it would have on seed dispersal.

Many questions remain open regarding the cognitive aspects that might influence the seed dispersal process. Experiment 1 could be extended, testing tortoises in more complicated spatial learning tasks to understand more in depth the mechanisms behind their navigation skills. In particular it would be interesting to verify whether tortoises are able to optimize the route between food sources and also to reorganize their path according to the position of the best food source. This particular skill has been investigated in different animal species (birds: Gill 1988; primates: Cramer and Gallistel 1997; Di Fiore and Suarez 2007; bees: Lihoreau *et al.*, 2011; rodents: Reid and Reid 2005) but not in reptiles, and revealed to be adaptive since allows animals to undertake the shortest possible route to visit multiple resources, saving therefore energy (Lihoreau *et al.*, 2011). This information would help to potentially predict the route of tortoises once the position of food sources is known: if tortoises prioritize high quality food patches by starting to forage from them, this means that the location of high quality resources could influence the fruit removal sequence and rate in a determined area. Moreover, knowing if tortoises establish a preferred route would potentially allow predicting defecation sites and, consequently, seed deposition patterns. However, this approach has its limits: although experiments in laboratory are essential to understand the mechanisms that regulate animals' decision process, they are far from the possible scenarios found in nature. As a consequence,

although cognitive studies reveal the potential of animals' skills, this not necessarily means that animals use these skills in their natural environment. Thus, it is essential to add to the laboratory findings observations in the wild. The use of radio-tracking techniques would be useful to test whether red-footed tortoises tend to return to the same food sources regularly between fruiting seasons. To date tracking data on red-footed tortoises' movements provide information about the distance travelled per day (Strong and Fragoso, 2006; Gùzman and Stevenson, 2008; Montaña *et al.*, 2013), without giving detailed information about the direction of the movement, and in particular about the conformation of the landscape and plants of the areas visited more frequently (if any). However, such information would be useful to validate the seed dispersal model comparing it with the actual movement of tortoises and defecation pattern.

#### **6.1.2 What? How animals feeding choices affect seed dispersal**

The third chapter of the thesis investigated whether frugivores' discriminatory skills and memory affect their choice of fruit. Red-footed tortoises were tested in a two choice task in which they had to choose between stimuli representing different quantities or qualities of food located at different distances (experiment 3). In the next experiment (experiment 4), the tortoises were tested in the same task after a retention time interval of 18 months, in order to investigate their long-term memory in a discrimination task. Tortoises were able to successfully discriminate between the visual cues indicating different types of food, and they included the cost (distance) in the decision process: they chose larger over smaller quantities no matter the distance, but only chose preferred over

non-preferred food when this was closer or at equal distance. This suggests that some characteristics of food can motivate frugivores to choose a certain food source more than others. The tortoises were, in fact, willing to make a greater effort to have a larger quantity of food than a preferred one. Moreover, tortoises retained the information about the food quality and quantity for a period of 18 months, remembering the association of the visual cue – and reward type. This suggests that frugivores can remember features indicating the presence of a food source for substantial periods of time. Thus, this might affect their long-term foraging decisions, which may result in different seed dispersal effects.

A seed dispersal model designed to test the extent of memory of discriminatory skills on the seed dispersal was presented (experiment 5). Results showed that quality and quantity are important features in influencing animal's foraging choice, as it preferred to forage from the plant in the environment that was offering the better food. However, results were very different depending on the memory skills of the animals: (1) the amount of seeds dispersed in the environment increased exponentially at higher level of memory, showing that the ability to remember the features of the food sources, and base the foraging decision on previous learned experiences, significantly increased fruit removal rate of the preferred fruit. (2) Only animals with longer memory produced an inequality in the amount of seeds dispersed from the two plants in the environment. This means that only animals that actually remember about the differences between the food sources were able to produce a significant difference in terms of seed removal, favouring the higher values plants. (3) For the same reasons as in chapter 2, animals with longer memory dispersed the seeds at shorter

distances from the parental plant than animals with shorter memory, they also survived longer.

These findings show, once again, how information about the cognition of frugivores adds a further step in the understanding of animals foraging decisions and seed dispersal. Without memory the animal would be forced to gain experience about the food at every feeding event, thus, establishing its choices on its perception of food rather than its experience of food. This would be time consuming for the animal, and affect seed dispersal: the model showed that plants that offer the preferred food would receive higher seed removal rate only if frugivores remember about their features. In the opposite case - frugivores do not remember about the food of the plant previously visited - the removal rate would depend on the chances of an animal to encounter and feed on the plants. In this scenario, the location of the plants in respect to other food sources (Carlos and Morales, 2008) and their attractiveness (colours and scents) (Schaefer *et al.*, 2004) would play a much more essential role.

Thus, further research regarding what motivates frugivores to choose certain fruits instead of other, and how long they remember about it, is essential to understand their foraging activity and the consequent removal rate. Experiment 3 and 4 could be extended by looking at factors, other than food itself, that might attract frugivores. In particular, it would be interesting to test whether tortoises might use acoustic cues, as well as olfactory ones, to locate food sources. Tortoises are ground dwelling animals, therefore it might be possible that they use monkeys and birds' vocalization or the noise of a fallen fruit, to locate potential food sources. Monkeys usually forage in groups, moving trees branches and

discarding some fruits (Janson, 1990; Suarez, 2014). Their foraging behaviour is likely to create valuable food sources on the ground that can be used by other animals. Plants would also benefit from this behaviour because their seeds would be removed from under parental trees, where the mortality is higher, and their seed shadow would be expanded.

### **6.1.3 When? How anticipatory behaviour impact on seed dispersal**

Chapter 4 examined whether frugivores' ability to anticipate events, such as food availability, would benefit seed dispersal and improve the foraging efficiency of animals: plants would receive fast seed removal when the fruits are ready to be eaten and frugivores would avoid visiting plants without fruits, directing their foraging towards profitable areas of the landscape.

Therefore, the ability of the red-footed tortoise to anticipate food delivery time was tested on a 24h cycle (experiment 6). Then, the hypothesis that the ability to anticipate fruiting periods of plants can increase the amount of seeds dispersed in the environment and the foraging efficiency of frugivores was tested using the seed dispersal model (experiment 7).

Tortoises were able to successfully anticipate the food delivery event on a 24 h cycle. Their activity increased in the hour prior the scheduled food delivery time. This behaviour extinguished in three days after food was presented at a different time, showing strong evidence of behavioural flexibility. This is likely to be adaptive as it prevents tortoises to return to the same food source once it is depleted (Moore *et al.*, 2011). Results of the model suggested that if animals have a memory that is at least long as the duration of a plant fruiting cycle, they would



increase exponentially the amount of seed dispersed in the environment. This means that a long-term anticipatory skill would increase animals' efficiency in finding food. However, the survival rate of animals was due to memory in the same way as in the models explained in the previous chapters, meaning that animals with longer memory survive longer thanks to their better ability of re-locating the food sources, but this did not interact with the length of plant cycle.

Results of chapter 4 suggests that whether frugivores use their anticipatory skills to plan their foraging would have big impact on the amount of seed removal from plants. Animals able to anticipate plants' fruiting periods are better in relocating the plant when it is fruiting, and these non-random feeding events (1) increase the number of seeds dispersed in the environment, and (2) allow the prediction of frugivores movements. As a consequence, it might be possible to predict how the seed shadow of plants varies according to the different fruiting seasons of plants. However, although it is known that frugivores have the potential to anticipate food delivery events (e.g. frugivores primates and birds: Waite *et al.*, 2001; Feeney *et al.*, 2011, and the present study on red-footed tortoise) whether they possess a "time map" of the fruiting periods of different plants in the environment they inhabit is unknown. A study on garden warblers showed that they were able of anticipating food delivery events in different rooms, where food was available at different time of the day - but always on 24h cycle - thereby being in the right position at the right moment (Biebach *et al.*, 1989). Thus, it might be possible that frugivores possess the same skill, thus being potentially able to anticipate different fruiting period of plants and directing their foraging accordingly.

Further studies are necessary to understand about anticipatory skills of frugivores, in particular as regard periods that are longer than 24 h cycle, as the intervals between plants' fruiting cycle can be several months long (Moskovitz and Bjørndal, 1990). Experiment 6 could be extended testing the anticipatory skill of tortoises, and/or other frugivores, on food delivery cycles longer than 24 h, for example delivering a particularly preferred type of food in a specific location once a week, in order to verify whether they are able of anticipating events on a long time scale, in order to understand if the hypothesis tested in the model (experiment 7) would actually be possible.

#### **6.1.4 Tortoises' model**

Chapter 5 presented a model parameterised with red-footed tortoise data. The models in the previous chapters showed the impact that animals with different range of memory would have on seed dispersal. This last model wanted to test one of the characteristics that makes tortoises unusual as seed dispersal vectors: the use of gaps in the forest. Specifically, the aim was to test the seed dispersal impact of frugivores that actively use gaps in different types of environment, i.e. closed canopy and deforested landscape, in comparison with frugivores that use gap only by chance (experiment 8).

The results showed that animals that use gaps, such as tortoises, disperse a significantly greater number of seeds in forest gaps, in both closed canopy and deforested environments, than animals that visit gaps at random. These results suggest that tortoises might potentially be used in the restoration of the environments, in particular the ones that suffered deforestation. Reforestation of

large areas disturbed by humans' activity is often dispersal limited: most plant species are very slow to arrive, partially because of the absence of seed dispersers in those areas (Howe and Miriti, 2004). A common solution adopted by human to overcome dispersal limitation in deforested areas is to place perches in fields to attract birds and their loads of seeds (Miriti 1998, Holl 1999), or to plant short-lived trees to encourage shade-tolerant tree seedlings and suppress competition from grasses (Hooper *et al.*, 2002). These precautions promote the movements of pollinators and dispersal agents in deforested areas (Tewksbury *et al.*, 2002) favoring the arrival of seeds through frugivores, and accelerating the growth of buffers and corridors that have a positive feedback on further frugivores arrival (Howe and Miriti, 2004). Tortoises, because of their thermoregulatory needs, are more prone than other seed disperser species to visit areas without any forest cover. This behaviour would potentially deliver the same advantages brought by human intervention. Thus they might be use as reforestation tool.

## **6.2 Conclusion**

This thesis took a first step in exploring whether understanding the cognitive mechanisms underlying frugivores' foraging behaviour would bring new insight, and help to build more reliable predictions of seed dispersal by endozoochory. The results obtained indicate that cognition is an important factor in the understanding of seed dispersal process. The cognitive approach could, in fact, provide new insights in the study of seed dispersal. As stated by Cousens *et al.*, 2010, the possible key to a deeper understanding of seed dispersal by endozoochory is to address the attention on what determines the behaviour of the

animals, instead of limiting the simulation to a behaviour of the vector in a specific case of study, as usually done in this work. This thesis looked into this hypothesis concluding that an examination of the cognitive processes underneath frugivores' foraging behaviour might lead to predictions about seed dispersal that would not be discovered only from the analysis of animal behaviour. Thus, cognition, informed by behavioural ecology, is probably the most effective way to understand and predict an animal's choices and movements around the environment, therefore additional research in this direction is essential to fully understand the seed dispersal process by endozoochory.

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